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(71) Applicant *(for all designated States except US)*: VIMRX HOLDINGS, LTD. [US/US]; Suite 210, 2751 Centerville Road, Little Falls II, Wilmington, DE 19808 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LUDWIG, János [DE/DE]; Herzberger Landstrasse 43 b, D-37085 Göttingen (DE). SPROAT, Brian [GB/DE]; Am Antonsberg 10, D-37139 Adelebsen (DE).

(74) Agents: WEICKMANN, H. et al.; Kopernikusstrasse 9, D-81679 Munich (DE).

(54) Title: CHIMERIC OLIGOMERS HAVING AN RNA-CLEAVAGE ACTIVITY

(57) Abstract

New chimeric oligomers having an RNA-cleavage activity, as well as their use for cleaving RNA-substrates in vitro and in vivo have been disclosed. The new chimeric oligomers contain an active centre, the building blocks of which are selected from nucleotides or/and nucleotide analogues, as well as flanking regions contributing to the formation of a specific hybridization of the oligomer with an RNA-substrate and containing at least one building block which is different from deoxyribonucleotides and ribonucleotides.

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Chimeric oligomers having an RNA-cleavage activity

Description

The present invention concerns chimeric oligomers having an RNA-cleavage activity and the use thereof for the cleavage of RNA substrates in vitro and in vivo.

Hammerhead ribozymes are an example of catalytic RNA molecules which are able to recognize and cleave a given specific RNA substrate (Hotchins et al., Nucleic Acids Res. 14 (1986), 3627; Keese and Symons in: Viroids and viroid - like pathogens, J.J. Semanchik, Publ. (CRC-Press, Boca Raton, Florida, (1987), p. 1 - 47). The catalytic centre of hammerhead ribozymes contains three stems and can be formed by adjacent sequence regions of the RNA or also by regions which are separated from one another by many nucleotides. Fig 1 shows a diagram of such a catalytically active hammerhead structure. The stems have been denoted I, II and III. The nucleotides are numbered according to the standard nomenclature for hammerhead ribozymes (Hertel et al., Nucleic Acids Res. 20 (1992), 3252).

The consensus sequence of the catalytic core structure is described by Ruffner and Uhlenbeck (Nucleic Acids Res. 18 (1990), 6025-6029). Perriman et al. (Gene 113 (1992), 157 - 163) have meanwhile shown that this structure can also contain variations e.g. naturally occurring nucleotide insertions such as $N^{9\lambda}$ and $N^{\lambda 12}$, or modifications that can be tolerated such as $R^{15.1}$=G. Thus the positive strand of the satellite RNA of the tobacco ring-spot virus does not contain any of the two nucleotide insertions while the +RNA strand of the virusoid of the lucerne transient streak virus (vLTSV) contains a $N^{9\lambda}$ = U insertion which can be mutated to C or G without loss of activity (Sheldon and Symons, Nucleic Acids Res. 17 (1989), 5679-5685). Furthermore in this special case N^{7} = A and $R^{15.1}$ = A. On the other hand the minus strand of the carnation stunt associated viroid (- CarSV) is quite unusual since it contains both nucleotide

insertions i.e. $N^{\lambda 12}$ = A and $N^{9\lambda}$ = C (Hernandez et al., Nucleic Acids Res. 20 (1992), 6323-6329). In this viroid N^{7} = A and $R^{15.1}$ = A. In addition this special hammerhead structure exhibits a very effective self-catalytic cleavage despite the more open central stem.

The possibilities of using hammerhead RNA enzymes range from the isolation of RNA restriction enzymes to the specific inactivation of the expression of genes in cells e.g. in animal, human or plant cells and in prokaryotes, yeasts and plasmodia. A particular biomedical interest is based on the fact that many diseases, including many forms of tumours, are related to the expression of specific genes. Inactivating such genes by cleaving the associated mRNA could represent a possible way to control and eventually treat such diseases. Moreover there is a great need to develop antiviral or antimicrobial pharmaceutical agents, the RNA enzymes possibly being such an agent, since viral expression can be blocked selectively by cleaving viral or microbial RNA molecules.

The most promising variants of pharmaceutical agents for the specific inactivation of the expression of genes are modified oligonucleotides which contain a block of deoxyribonucleotides in the middle region of the molecule (Giles et al., Nucleic Acids Res. 20 (1992), 763 - 770). These deoxyribonucleotides form a DNA-RNA hybrid with RNA the RNA strand of which is cleaved by cellular RNAse H. However, a disadvantage of these oligonucleotides for in vivo applications is their low specificity, since hybrid formation and thus cleavage can also take place at undesired positions on the RNA molecules. In addition the DNA sequences interact undesirably with cellular proteins.

Previous attempts to recombinantly express catalytically active RNA molecules of the cell by transfecting the cell with an appropriate gene have not proven to be very effective since a very high expression was necessary to inactivate specific RNA substrates. In addition the vector systems which are available now cannot generally be applied. Furthermore unmodified RNA

enzymes cannot be administered directly due to the sensitivity of RNA to degradation by RNAses and their interactions with proteins.

Thus chemically modified active substances have to be administered in order to administer hammerhead ribozymes exogenously (cf. e.g. Heidenreich et al., J. Biol. Chem. 269 (1994), 2131-2138; Kiehntopf et al., EMBO J. 13 (1994), 4645-4652; Paolella et al., EMBO J. 11 (1992), 1913-1919 and Usman et al., Nucleic Acids Symp. Ser. 31 (1994), 163-164).

DE-OS 42 16 134 describes such chemically modified active substances based on synthetic catalytic oligonucleotide structures with a length of 35-40 nucleotides which are suitable for cleaving a nucleic acid target sequence and contain modified ribonucleotides that contain an optionally substituted alkyl, alkenyl or alkynyl group with 1 - 10 carbon atoms at the 2'-O atom of the ribose. Furthermore oligonucleotides are described which contain the above-mentioned modified nucleotide building blocks and which form a hammerhead structure. These oligonucleotides are able to cleave specific RNA substrates. Examples of oligonucleotides are described having an active centre which has a length of 14 nucleotides. This active centre contains several ribonucleotides that increase the sensitivity towards enzymes which cleave RNA. A further disadvantage is the length of the active centre which can often lead to unspecific hybridization.

WO 95/11304 describes RNA-cleaving nucleic acids with an active centre that is free of ribonucleotide building blocks but instead contains deoxyribonucleotides. However, the deoxyribonucleotides used in the active centre result in a very low RNA cleavage activity. Thus it was reported that a 13-mer deoxyribozyme of the "GAAA" type based on LTSV was not able to cleave a 41-mer oligoribonucleotide substrate while the corresponding 13-mer ribozyme exhibited an excellent catalytic activity (Jeffries and Symons, Nucleic Acids Res. 17 (1989), 1371-1377).

In addition the use of a larger number of deoxyribonucleotide

building blocks in the hybridization arms or in the active centre leads to loss of specificity due to an activation of RNAse H since sequences which are related to the desired target sequence can also be cleaved. Moreover catalytic DNA oligomers are not particularly well suited for in vivo applications due to interactions with proteins.

The object of the present invention was therefore to provide chimeric oligomers that cleave RNA in which the disadvantages of the state of the art are at least partially eliminated. In particular it is intended to provide chimeric RNA-cleaving oligomers which at the same time have a high stability and a high effectivity and specificity as well as being suitable for in vivo applications.

This object is achieved by a chimeric oligomer having RNA cleavage activity which has a structure of formula (I):

5'-X-Z-Y-3' (I)

in which X and Y represent oligomeric sequences containing building blocks composed of nucleotides or/and nucleotide analogues and contribute to the formation of a specific hybridization of the oligomer with an RNA substrate,

in which X and Y each contain at least one building block that differs from deoxyribonucleotides and ribonucleotides

in which Z represents the active centre of the chimeric oligomer having a structure of formulae (IIIa), (IIIb), (IVa) or (IVb):

$5'\text{-}G^{12}\text{-}A^{13}A^{14}R^{15.1}\text{-}3'$ (IIIa)

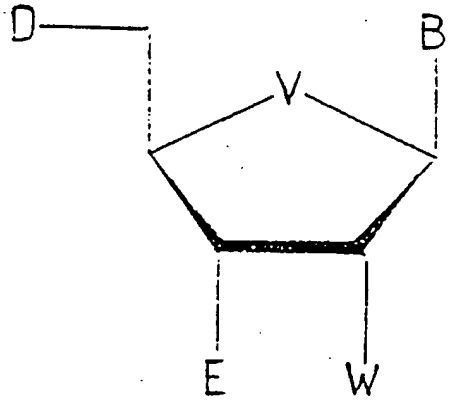
$5'\text{-}N^{\lambda 12}G^{12}A^{13}A^{14}R^{15.1}\text{-}3'$ (IIIb)

$5'\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}\text{-}3'$ (IVa)

$5'\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}N^{9\lambda}\text{-}3'$ (IVb)

in which the building blocks of the structures (IIIa), (IIIb), (IVa), (IVb) are selected from nucleotides or/and nucleotide analogues

in which the building blocks have a structure of formula (II):



(II)

in which B denotes a residue which is a naturally occurring nucleobase or a functional equivalent thereof, in particular selected from the group comprising adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), uracil-5-yl (ψ), hypoxanthin-9-yl (I), thymin-1-yl (T), 5-methylcytosin-1-yl (5MeC), 2,6-diaminopurin-9-yl (aminoA), purin-9-yl (P), 7-deazaadenin-9-yl (c^7A), 7-deazaguanin-9-yl (c^7G), 5-propynylcytosin-1-yl (5-pC), 5-propynyluracil-1-yl (5-pU), isoguanin-9-yl, 2-aminopurin-9-yl, 6-methyluracil-1-yl, 4-thiouracil-1-yl, 2-pyrimidone-1-yl and quinazoline-2,4-dione-1-yl;

V independently denotes an O, S, NH or CH_2 group
W independently denotes an H or OH or a straight-chained or branched alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy with 1 to 10 C atoms which can be optionally substituted by one or several halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxyl or/and mercapto groups or is selected from -COOH, $-CONH_2$, $-CONHR^1$, $-CONR^1R^2$, $-NH_2$, $-NHR^1$, $-NR^1R^2$, $-NHCOR^1$, -SH, $-SR^1$, -F, $-ONH_2$, $-ONHR^1$, $-ONR^1R^2$, -NHOH, $-NHOR^1$, $-NR^2OH$ and NR^2OR^1 in which R^1 and R^2 independently denote unsubstituted or substituted alkyl, alkenyl or alkynyl groups as defined above,
D and E denote residues which together form a phosphodiester or

phosphorothioate diester bond between adjacent nucleosides or analogues or together form an analogue of an internucleosidic bond,

in which the structures (IIIa) or (IIIb) do not contain a total of more than two deoxyribonucleotides and the structures (IVa) or (IVb) do not contain a total of more than four deoxyribonucleotides and

in which G in each case independently denotes a building block according to formula (II) in which B is guanin-9-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from I, c^{7}G and isoguanin-9-yl;

A in each case independently denotes a building block according to formula (II) in which B is adenin-9-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from aminoA, P, c^{7}A and 2-aminopurin-9-yl;

U in each case independently denotes a building block according to formula (II) in which B is uracil-1-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from ψ, T, 5-pU, 6-methyluracil-1-yl, 4-thiouracil-1-yl, 2-pyrimidone-1-yl and quinazoline-2,4-dione-1-yl;

C in each case independently denotes a building block according to formula (II) in which B is cytosin-1-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from 5MeC, 5-pC and 2-pyrimidone-1-yl;

R in each case independently denotes a building block according to formula (II) in which B independently denotes G or A as defined

above,

N in each case independently denotes a building block according to formula (II).

The monomeric building blocks of sequences X and Y which flank the active centre are nucleotide or/and nucleotide analogues and are selected in such a way that they specifically hybridize with a given RNA substrate and, together with the active centre Z, form a structure, especially a hammerhead structure which specifically cleaves the RNA substrate.

The nucleotide building blocks can, on the one hand, be selected from ribonucleotides. However, the number of ribonucleotides should be as small as possible since the presence of ribonucleotide building blocks strongly reduces the in vivo stability of the oligomers. The flanking regions X and Y (and also the active centre Z) should in particular not contain any natural ribonucleotide building blocks at the positions taken up by pyrimidine nucleotides but rather modified building blocks.

The use of a larger number of deoxyribonucleotides for the flanking sequences X and Y is also less preferred since undesired interactions with proteins can occur or the formation of an RNAse H-sensitive DNA-RNA hybrid. Thus the flanking sequences each preferably contain at most 1 and particularly preferably no ribonucleotide at all and a consecutive sequence of a maximum of 3 deoxyribonucleotides and especially no more than 2 deoxyribonucleotides.

The building blocks of the flanking sequences X and Y are preferably selected from nucleotides or/and nucleotide analogues e.g. from building blocks having the structure (II) which are also used for the active centre Z. Preferred modified building blocks are those in which W is in each case independently selected from optionally substituted alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy residues with 1 - 10 C atoms. Particularly preferred substituents are for example methyl, ethyl, propyl, allyl, methoxy, ethoxy, propoxy, allyloxy, hy-

droxyethyloxy and methoxyethyloxy. However, other modified building blocks can also be used such as nucleotide analogues which contain amino groups, substituted amino groups, halogen groups or sulfhydryl groups at the 2' position of pentose.

The internucleosidic linkage between two nucleoside building blocks can be achieved by phosphodiester bonds or by modified phospho bonds such as by phosphorothioate groups or other bonds such as for example those described in DE-OS 42 16 134.

In addition the flanking regions X and Y can also contain nucleotide analogues such as peptidic nucleic acids (see for example Nielsen et al., Science 254 (1991), 1497-1500 and Dueholm et al., J. Org. Chem. 59 (1994), 5767-5773). In this case the linkage of individual building blocks can for example be achieved by acid amide bonds. The linkage of flanking regions X and Y which are based on peptidic nucleic acids with an active centre Z based on nucleotide building blocks can either be achieved by suitable linkers (see e.g. Petersen et al., BioMed. Chem. Lett. 5 (1995), 1119-1121) or directly (Bergmann et al., Tetrahedron Lett. 36 (1995), 6823-6826.

The flanking regions X and Y preferably contain independently of each other 3 - 40 and particularly preferably 5 - 10 nucleotide or nucleotide analogue building blocks.

The building blocks of the flanking regions X and Y contain nucleobases or analogues which can hybridize with bases that occur naturally in RNA molecules. The nucleobases are preferably selected from naturally occurring bases (adenine, guanine, cytosine, thymine and uracil) as well as analogues thereof e.g. 2,6-diaminopurine, hypoxanthine, 5-methylcytosine, pseudouracil, 5-propynyluracil, 5-propynylcytosine etc. which enable a specific binding to the target RNA.

A firm binding to the RNA substrate is preferred in the regions X and Y so that the following nucleobases are particularly preferred as building blocks: 2,6-diaminopurine instead of adeni-

ne; thymine or 5-propynyluracil instead of uracil; 5-methylcytosine or 5-propynylcytosine instead of cytosine. In addition 2-amino-2'-O-alkyladenosines are preferred (Lamm et al., Nucleic Acids Res. 19 (1991), 3193-3198). Furthermore aromatic systems can be linked to positions 4 and 5 of uracil, such as B = phenoxazine by which means dramatic improvements are obtained in the stability of the double-strand (Lin et al., J. Am. Chem. Soc. 117 (1995), 3873-3874).

The 3' end of the oligomer according to the invention can additionally be protected against degradation by exonucleases for example by using a modified nucleotide building block that is also modified at the 3' position of the ribose sugar e.g. by an optionally substituted alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy group as defined above. An additional possibility of further stabilizing oligomers according to the invention at the 3' end against degradation by exonucleases is a 3'-3'-linked dinucleotide structure or/and the presence of two modified phospho bonds such as two phosphorothioate bonds.

Moreover the chimeric oligomer structure according to the invention can be linked to a prosthetic group in order to improve its cellular uptake or/and to enable a specific cellular localization. Examples of such prosthetic groups are polyamino acids (e.g. polylysine), lipids, hormones or peptides. These prosthetic groups are usually linked via the 3' or 5' end of the oligomer either directly or by means of suitable linkers (e.g. linkers based on 6-aminohexanol or 6-mercapto-hexanol etc.). These linkers are commercially available and techniques suitable for linking prosthetic groups to the oligomer are known to a person skilled in the art.

The increase in the rate of hybridization is particularly important for the biological activity of the oligomers according to the invention since in this way it is possible to achieve a high activity at low concentrations. This is particularly important for short-lived RNA substrates or RNA substrates that occur less often. A substantial acceleration of the hybridiza-

tion can for example be achieved by coupling positively charged peptides, which for example contain several lysine residues, to the end of an oligonucleotide (Corey, J. Am. Chem. Soc. 117 (1995), 9373-9374). A chimeric oligomer according to the invention can be simply modified in this manner using the linkers described above. Alternatively the rate of hybridization can also be increased by incorporation of building blocks which contain sperminyl residues (Schmid and Behr, Tetrahedron Lett. 36 (1995), 1447 - 1450). Such modifications of the chimeric oligomers also improve the ability to bind to RNA substrates having secondary structures.

The active centre Z of the chimeric oligomer according to the invention contains nucleotide building blocks which are modified and optionally a small number of natural nucleotides i.e. ribonucleotides or/and deoxyribonucleotides.

The nucleotide building blocks in the region Z are preferably selected from compounds which can only hybridize weakly with ribonucleotides such as modified nucleotides that contain an optionally substituted alkyl, alkenyl or alkynyl group with preferably 1-5 C atoms at the 2'-C atom of ribose. In addition substituents are also preferred which are selected from optionally substituted alkoxy, alkenyloxy or alkynyloxy groups with 1-5 C atoms. Particularly preferred nucleobases in the region Z are A or P, U, C and G or I.

According to a first embodiment of the present invention the active centre Z of the chimeric oligomer contains a structure of formulae (IIIa) or (IIIb). Such an oligomer can cleave an RNA substrate which contains a structure of formula (Va) or (Vb):

$$5'\text{-}U^{16.1}H^{17}\text{-}S\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}R^{10.1}\text{-}3' \qquad \text{(Va)}$$

$$5'\text{-}U^{16.1}H^{17}\text{-}S\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}N^{9A}R^{10.1}\text{-}3' \qquad \text{(Vb)}$$

in which N, H, R, S, G, A, U and C represent ribonucleotides and

N denotes G, A, U or C;
H denotes A, C or U;
R denotes A or G and
S is an RNA sequence capable of forming a hairpin structure with a length of preferably 6 - 60 and particularly preferably of 6 - 20 bases.

An oligomer with an active centre (IIIa) or (IIIb) can form the hammerhead structure shown in Fig. 2 with an RNA substrate containing a structure of formulae (Va) or (Vb) in which the nucleotides are numbered according to the standard nomenclature. The active centre Z (IIIa) or (IIIb) preferably contains no more than four and particularly preferably no more than two ribonucleotides and most preferably no more than one ribonucleotide.

Particularly good results were obtained for oligomers in which G12 is a deoxyribonucleotide, a ribonucleotide or a building block in which W is a C_1-C_4 alkyl, alkenyl, alkoxy or alkenyloxy group optionally substituted by OH and in particular a (2-hydroxyethyloxy) group. A^{13}, A^{14} and/or $R^{15.1}$ are preferably selected from building blocks in which W is preferably a C_1-C_4 alkyl, alkenyl, alkoxy or alkenyloxy group optionally substituted by OH and particularly preferably a methoxy, (2-hydroxy-ethyloxy) and allyloxy group. In addition it was also possible to synthesize an oligomer which does not contain <u>any</u> ribonucleotide and <u>any</u> deoxyribonucleotide in the active centre Z. This oligomer contains a 2'-O-(2-hydroxyethyl)guanosine at position G^{12} and a 2'-O-(2-hydroxyethyl)adenosine at each of the positions A^{13}, A^{14} and $R^{15.1}$.

According to a second embodiment of the present invention the active centre of the oligomer has a structure of formulae (IVa) or (IVb) and exhibits cleavage activity for an RNA substrate that contains a structure of formula (VIa) or (VIb):

$$5'\text{-}Y^{11.1}G^{12}A^{13}A^{14}R^{15.1}\text{-}S\text{-}U^{16.1}H^{17}\text{-}3' \qquad \text{(VIa)}$$

$$5'\text{-}Y^{11.1}N^{\lambda 12}G^{12}A^{13}A^{14}R^{15.1}\text{-}S\text{-}U^{16.1}H^{17}\text{-}3' \qquad \text{(VIb)}$$

in which N, H, Y, R, S, G, A, U and C represent ribonucleotides and

N denotes G, A, U or C
H denotes A, C or U
R denotes A or G
Y denotes C or U and
S is an RNA sequence capable of forming a hairpin structure with a length of preferably 6 - 60 and particularly preferably of 6 - 20 bases.

An oligomer with an active centre (IVa) or (IVb) forms the hammerhead structure shown in Fig. 3 with an RNA substrate containing a structure of formulae (VIa) or (VIb) in which the nucleotides are numbered according to the standard nomenclature. The active centre Z preferably contains no pyrimidine ribonucleotides. Z preferably contains no more than six and particularly preferably no more than three ribonucleotides.
If the active centre Z contains ribonucleotides, one or several building blocks at positions G^5, A^6, N^7 (if N^7 is a purine), G^8, A^9 and N^{9A} (if N^{9A} is present and is a purine) are thus preferably ribonucleotides. The building blocks at positions C^3, U^4 N^7 (if N^7 is a pyrimidine) and N^{9A} (if N^{9A} is present and is a pyrimidine) are preferably nucleotide analogue building blocks in particular such building blocks in which W is an optionally OH-substituted C_1-C_4 alkyl, alkenyl, alkoxy or alkenoxy group or an NH_2 group. Building blocks are especially preferred which can only hybridize very weakly with ribonucleotides in the substrate such as those nucleotide analogues which contain an optionally substituted alkyl, alkenyl or alkynyl group according to formula (II), e.g. an allyl group, at the 2' C atom of the ribose. However, 2'-O-nucleotide analogues are on the other hand also preferred. Specific examples of suitable substituents are methoxy, (2-hydroxyethyloxy), allyl, allyloxy and NH_2. In addition N^7 and N^{9A}, if they are present, are selected in such a way that a minimum of internal structures can form by internal interactions. The preferred nucleobases in the region Z are A

N denotes G, A, U or C;
H denotes A, C or U;
R denotes A or G and
S is an RNA sequence capable of forming a hairpin structure with a length of preferably 6 - 60 and particularly preferably of 6 - 20 bases.

An oligomer with an active centre (IIIa) or (IIIb) can form the hammerhead structure shown in Fig. 2 with an RNA substrate containing a structure of formulae (Va) or (Vb) in which the nucleotides are numbered according to the standard nomenclature. The active centre Z (IIIa) or (IIIb) preferably contains no more than four and particularly preferably no more than two ribonucleotides and most preferably no more than one ribonucleotide.

Particularly good results were obtained for oligomers in which G12 is a deoxyribonucleotide, a ribonucleotide or a building block in which W is a C_1-C_4 alkyl, alkenyl, alkoxy or alkenyloxy group optionally substituted by OH and in particular a (2-hydroxyethyloxy) group. A^{13}, A^{14} and/or $R^{15.1}$ are preferably selected from building blocks in which W is preferably a C_1-C_4 alkyl, alkenyl, alkoxy or alkenyloxy group optionally substituted by OH and particularly preferably a methoxy, (2-hydroxy-ethyloxy) and allyloxy group. In addition it was also possible to synthesize an oligomer which does not contain <u>any</u> ribonucleotide and <u>any</u> deoxyribonucleotide in the active centre Z. This oligomer contains a 2'-O-(2-hydroxyethyl)guanosine at position G^{12} and a 2'-O-(2-hydroxyethyl)adenosine at each of the positions A^{13}, A^{14} and $R^{15.1}$.

According to a second embodiment of the present invention the active centre of the oligomer has a structure of formulae (IVa) or (IVb) and exhibits cleavage activity for an RNA substrate that contains a structure of formula (VIa) or (VIb):

$$5'\text{-}Y^{11.1}G^{12}A^{13}A^{14}R^{15.1}\text{-}S\text{-}U^{16.1}H^{17}\text{-}3' \qquad \text{(VIa)}$$

$$5'\text{-}Y^{11.1}N^{\lambda 12}G^{12}A^{13}A^{14}R^{15.1}\text{-}S\text{-}U^{16.1}H^{17}\text{-}3' \qquad \text{(VIb)}$$

in which N, H, Y, R, S, G, A, U and C represent ribonucleotide and

N denotes G, A, U or C
H denotes A, C or U
R denotes A or G
Y denotes C or U and
S is an RNA sequence capable of forming a hairpin structure with a length of preferably 6 - 60 and particularly preferably of 6 - 20 bases.

An oligomer with an active centre (IVa) or (IVb) forms the hammerhead structure shown in Fig. 3 with an RNA substrate containing a structure of formulae (VIa) or (VIb) in which the nucleotides are numbered according to the standard nomenclature. The active centre Z preferably contains no pyrimidine ribonucleotides. Z preferably contains no more than six and particularly preferably no more than three ribonucleotides. If the active centre Z contains ribonucleotides, one or several building blocks at positions G^5, A^6, N^7 (if N^7 is a purine), G^8, A^9 and N^{9A} (if N^{9A} is present and is a purine) are thus preferably ribonucleotides. The building blocks at positions C^3, U^4 N^7 (if N^7 is a pyrimidine) and N^{9A} (if N^{9A} is present and is a pyrimidine) are preferably nucleotide analogue building blocks in particular such building blocks in which W is an optionally OH-substituted C_1-C_4 alkyl, alkenyl, alkoxy or alkenoxy group or an NH_2 group. Building blocks are especially preferred which can only hybridize very weakly with ribonucleotides in the substrate such as those nucleotide analogues which contain an optionally substituted alkyl, alkenyl or alkynyl group according to formula (II), e.g. an allyl group, at the 2′ C atom of the ribose. However, 2′-O-nucleotide analogues are on the other hand also preferred. Specific examples of suitable substituents are methoxy, (2-hydroxyethyloxy), allyl, allyloxy and NH_2. In addition N^7 and N^{9A}, if they are present, are selected in such a way that a minimum of internal structures can form by internal interactions. The preferred nucleobases in the region Z are A

The minimum stability requirements for a hammerhead structure are three base pairs in stem I and three base pairs in stem III. Although all motifs which fulfil these minimum requirements can be cleaved, the cleavage efficiency depends on the interaction of the actual recognition sequences and on conserved nucleotides in the active centre.

Of the oligomers according to the invention the chimeric oligonucleotides containing GAAR seem to be less sensitive to interferences by undesired interactions and are therefore preferred.

RNA substrates of chimeric oligomers with an active centre which has a structure of formulae (IIIa) or (IIIb) are preferably human cellular transcripts and transcripts of human viruses. The RNA substrate is particularly preferably selected from the group comprising human interleukin-2 mRNA, human ICAM-1 mRNA, human TGF-ß mRNA, human tissue factor pre-mRNA, human protein kinase C-α mRNA, human factor KBF1 mRNA, human 5-lipoxygenase mRNA, human interleukin-8 RNA, human interleukin-5 RNA, human interleukin-1 receptor mRNA, human interleukin-1-α mRNA, human 12-lipoxygenase mRNA, the transcript of the human cytomegalovirus DNA polymerase gene and a transcript of the human papilloma virus type 8.

Particularly preferred cleavage motifs for oligomers of the "GAAR" type are located at the following positions of these RNA substrates (the name of the respective sequence in the EMBL Nucleotide Sequence Database 43rd Edition is given in brackets).

- human interleukin-2-mRNA (HSIL2R) with C^3 at position 490 and a cleavage after the sequence AUU;
- human ICAM-1 (intercellular adhesion molecule-1) mRNA (HSICAM01) with C^3 at position 1933 and a cleavage after the sequence AUU;
- human TGF-ß (transforming growth factor ß) mRNA (HSTGFB1) with C^3 at position 1313 and a cleavage after the sequence CUC;

- human tissue factor pre-mRNA (HSTFPB) with C^3 at position 6781 and a cleavage after the sequence GUU or with C^3 at position 8831 and a cleavage after the sequence AUC or CUU;
- human PKC-α (protein kinase C-α) mRNA (HSPKCA1) with C^3 at position 823 and a cleavage after the sequence UUC or UUU;
- human factor KBF-1 mRNA (HSNFKB34) with C^3 at position 2619 and a cleavage after the sequence UUA;
- human 5-lipoxygenase mRNA (HSLOX5A) with C^3 at position 296 and a cleavage after the sequence GUA;
- human interleukin-8 RNA (HSIL8A) with C^3 at position 4978 and a cleavage after the sequence AUA;
- human interleukin-5 RNA (HSIL5) with C^3 at position 1896 and a cleavage after the sequence AUU or UUU;
- human interleukin-1 receptor mRNA (HSIL1RA) with C^3 at position 1485 and a cleavage after the sequence AUC;
- human interleukin-1-α mRNA (HSIL1ALPH) with C^3 at position 1272 and a cleavage after the sequence CUU;
- human 12-lipoxygenase mRNA (HSLIPXYG) with C^3 at position 940 and a cleavage after the sequence AUA;
- transcript of the human cytomegalovirus DNA polymerase gene (HEHS5POL) with C^3 at position 3919 and a cleavage after the sequence UUA;
- transcript of the human papilloma virus type 8 (PAPPPH8C) with C^3 at position 3221 and a cleavage after the sequence CUU.

A particularly preferred example of a cleavage site in a human mRNA is interleukin-2 mRNA. The cleavage site has the nucleotide sequence shown in SEQ ID NO. 1. An oligomer of the "GAAR" type having the nucleotide sequence shown in SEQ ID NO. 2 is also able to efficiently cleave an appropriate RNA substrate. This oligomer according to the invention can also cleave a 1.6 kb long IL-2 transcript.

Human ICAM-1 mRNA is a further particularly preferred example of an RNA substrate which can be cleaved by the chimeric oligomers of the "GAAR" type. The region which is sensitive for

cleavage has the nucleotide sequence shown in SEQ ID NO. 3. An RNA substrate with this sequence can also be efficiently cleaved by an oligomer having the nucleotide sequence shown in SEQ ID NO. 4.

Human PKC-α mRNA is a further particularly preferred example of an RNA substrate, which can be cleaved by a chimeric oligomer of the "GAAR" type. The region which is sensitive to cleavage has the nucleotide sequence shown in SEQ ID NO. 9. This substrate is efficiently cleaved by an oligomer having the nucleotide sequence shown in SEQ ID. NO. 10.

Human interleukin-1α mRNA is a further particularly preferred example of an RNA substrate which can be cleaved by a chimeric oligomer of the "GAAR" type. The region which is sensitive to cleavage has the nucleotide sequence shown in SEQ ID NO. 11. This substrate is efficiently cleaved by an oligomer having the nucleotide sequence shown in SEQ ID NO. 12.

Preferred RNA substrates for oligomers according to the invention of the "CUGANGA" type are also human cellular transcripts and transcripts of human viruses. The RNA substrate is particularly preferably selected from the group comprising human interleukin-6 mRNA, human multiple drug resistance (MDR-1) mRNA, human monocyte chemotactic protein RNA, human macrophage scavenger receptor type II mRNA, human macrophage scavenger receptor type I mRNA, human macrophage inflammatory protein-1α mRNA, human p53 RNA, human jun-B mRNA, human c-jun RNA, human interferon
γ type II mRNA, human hepatocyte growth factor mRNA, human HER2 mRNA, human Alzheimer's disease amyloid mRNA, human interleukin-1 mRNA, human interleukin-1 receptor mRNA, human 3-hydroxy-3-methylglutaryl coenzyme A reductase RNA, human angiotensinogen mRNA, human angiotensin-converting enzyme mRNA, human acyl coenzyme A: cholesterol acyltransferase mRNA, human PDGF receptor mRNA, human TNF receptor mRNA, human TGF ß mRNA, human NF-kappa B p65 subunit mRNA, human c-myc RNA, human 12-lipoxygenase mRNA, human interleukin-4 RNA, human interleukin-10 mRNA,

human basic FGF mRNA, human EGF receptor mRNA, human c-myb mRNA, human c-fos RNA, human bcl-2 mRNA, human bcl-1 mRNA, human ICAM-1 mRNA, a transcript of human papilloma virus type 11 and transcripts of human papilloma virus type 16 and type 18.

Specific examples of suitable substrates are as follows:

- human monocyte chemotactic protein RNA (HSMCHEMP) with $Y^{11.1}$ at position 2183 and a cleavage after the sequence GUU;
- human macrophage scavenger receptor type II RNA (HSPHSR2) with $Y^{11.1}$ at position 833 and a cleavage after the sequence CUC;
- human macrophage scavenger receptor type I mRNA (HSPHSR1) with $Y^{11.1}$ at position 813 and a cleavage after the sequence CUC;
- human macrophage inflammatory protein 1-α mRNA (HSRANTES) with $A^{11.1}$ at position 629 and a cleavage after the sequence CUC;
- human p53 RNA (HSP53G) with $Y^{11.1}$ at position 19145 and a cleavage after the sequence GUC;
- human jun-B mRNA (HSJUNB) with $Y^{11.1}$ at position 357 and a cleavage after the sequence GUC;
- human C-jun RNA (HSJUNA) with $Y^{11.1}$ at position 3066 and a cleavage after the sequence CUC;
- human interferon-γ type II mRNA (HSIFNGAMM) with $Y^{11.1}$ at position 720 and a cleavage after the sequence GUU;
- human hepatocyte growth factor mRNA (HSHGF) with $Y^{11.1}$ at position 1197 and a cleavage after the sequence GUC or with $Y^{11.1}$ at position 1603 and a cleavage after the sequence GUA;
- human HER2 (tyrosine kinase receptor) mRNA (HSHER2A) with $Y^{11.1}$ at position 2318 and a cleavage after the sequence CUU;
- human Alzheimer's disease amyloid mRNA (HSAMY) with $Y^{11.1}$ at position 427 and a cleavage after the sequence AUC or with $Y^{11.1}$ at position 1288 and a cleavage after the sequence CUA;
- human interleukin-1 mRNA (HSIL1) with $Y^{11.1}$ at

position 113 and a cleavage after the sequence AUU;
- human interleukin-1 receptor (IL-1R) mRNA (HSIL1RA) with $Y^{11.1}$ at position 84 and a cleavage after the sequence CUC;
- human 3-hydroxy-3-methylglutarylcoenzyme A reductase mRNA (HSHMGCOB) with $Y^{11.1}$ at position 257 and a cleavage after the sequence CUC;
- human angiotensinogen mRNA (HSANG) with $Y^{11.1}$ at position 2012 and cleavage after the sequence UUC;
- human angiotensin-converting enzyme mRNA (HSACE) with $Y^{11.1}$ at position 1818 and a cleavage after the sequence GUA;
- human acyl coenzyme A: cholesterol acyltransferase mRNA (HSACYLCOA) with $Y^{11.1}$ at position 374 and a cleavage after the sequence CUA or with $Y^{11.1}$ at position 830 and a cleavage after the sequence GUA;
- human PDGF receptor mRNA (HSPDGFRA) with $Y^{11.1}$ at position 1513 and a cleavage after the sequence CUA;
- human tumour necrosis factor receptor mRNA (HSTNFRB) with $Y^{11.1}$ at position 502 and a cleavage after the sequence CUU or UUC;
- human TGF ß mRNA (HSTGFBC) with $Y^{11.1}$ at position 545 and a cleavage after the sequence AUC or with $Y^{11.1}$ at position 2428 and a cleavage after the sequence CUC;
- human NF kappa B p65 subunit mRNA (HSNFKB65A) with $Y^{11.1}$ at position 937 and a cleavage after the sequence UUC or with $Y^{11.1}$ at position 2195 and a cleavage after the sequence CUC;
- human c-myc RNA (HSMYCC) with $Y^{11.1}$ at position 4336 and a cleavage after the sequence CUU or with $Y^{11.1}$ at position 3799 and a cleavage after the sequence CUA;
- human c-myc mRNA (HSMYC1) with $Y^{11.1}$ at position 484 and a cleavage after the sequence CUU;
- human MDR-1 mRNA (HSMDR1) with $Y^{11.1}$ at position 1513 and a cleavage after the sequence AUA;
- human 12-lipoxygenase mRNA (HSLIPXYG) with $Y^{11.1}$ at position 566 and a cleavage after the sequence CUC;
- human interleukin-6 mRNA (HSIL6CSF) with $Y^{11.1}$ at

position 13 and a cleavage after the sequence CUA or with $Y^{11.1}$ at position 902 and a cleavage after the sequence GUA;

- human interleukin-4 RNA (HSIL4A) with $Y^{11.1}$ at position 2500 and a cleavage after the sequence GUC;
- human interleukin-10 mRNA (HSIL10) with $Y^{11.1}$ at position 1125 and a cleavage after the sequence CUU;
- human basic FGF mRNA (HSGFBF) with $Y^{11.1}$ at position 260 and a cleavage after the sequence CUA;
- human EGF receptor mRNA (HSEGFPRE) with $Y^{11.1}$ at position 4778 and a cleavage after the sequence AUA or with $Y^{11.1}$ at position 5508 and a cleavage after the sequence UUC;
- human c-myb mRNA (HSCMYBLA) with $Y^{11.1}$ at position 448 and a cleavage after the sequence GUU;
- human c-fos RNA (HSCFOS) with $Y^{11.1}$ at position 1065 and a cleavage after the sequence GUU or with $Y^{11.1}$ at position 3349 and a cleavage after the sequence UUU;
- human bcl-2 mRNA (HSBSL2C) with $Y^{11.1}$ at position 5753 and a cleavage after the sequence UUA;
- human bcl-1 mRNA (HSBCL1) with $Y^{11.1}$ at position 3725 and a cleavage after the sequence GUC;
- human ICAM-1 mRNA (HSICAM01) with $Y^{11.1}$ at position 1998 and a cleavage after the sequence GUA;
- a transcript of the human papilloma virus type 11 (PAPPPH11) with $Y^{11.1}$ at position 2941 and a cleavage after the sequence AUU;
- a transcript of the human papilloma virus type 16 (PA16) with $Y^{11.1}$ at position 37 and a cleavage after the sequence GUU;
- a transcript of the human papilloma virus type 16 (PA16) with $Y^{11.1}$ at position 1126 and a cleavage after the sequence UUA;
- a transcript of the human papilloma virus type 16 (PA16) with $Y^{11.1}$ at position 1322 and a cleavage after the sequence GUA;
- a transcript of the human papilloma virus type 16 (PA16) with $Y^{11.1}$ at position 1982 and a cleavage

after the sequence AUU;
- a transcript of the human papilloma virus type 16 (PA16) with $Y^{11.1}$ at position 6440 and a cleavage after the sequence UUA;
- a transcript of the human papilloma virus type 16 L1 protein gene (PAPHPU111) with $Y^{11.1}$ at position 99 and a cleavage after the sequence GUA;
- a transcript of the human papilloma virus type 18 (PAPPPH18) with $Y^{11.1}$ at position 896 and a cleavage after the sequence AUA;
- transcript of the human papilloma virus type 18 E6 protein gene (PARHPVE6) with $Y^{11.1}$ at position 172 and a cleavage after the sequence AUA.

A particularly preferred RNA substrate for a chimeric oligomer of the "CUGANGA" type is human interleukin-6 mRNA. The region which is sensitive to cleavage has the nucleotide sequence shown in SEQ ID NO. 5. An oligomer having the nucleotide sequence shown in SEQ ID NO. 6 can efficiently cleave such a substrate.

Yet a further particularly preferred example of an RNA substrate which can be cleaved by an oligomer of the "CUGANGA" type is human MDR-1 mRNA. The region which is sensitive to cleavage has the nucleotide sequence shown in SEQ ID NO. 7. An oligomer having the nucleotide sequence shown in SEQ ID No. 8 can efficiently cleave such an RNA substrate. A 1.6 kb long MDR-1 transcript can also be cleaved by this oligomer.

A further particularly preferred example of an RNA substrate which can be cleaved by an oligomer of the "CUGANGA" type is human c-jun RNA. The region which is sensitive to cleavage is shown in SEQ ID NO. 13. An oligomer having the nucleotide sequence shown in SEQ ID NO. 14 can cleave such a substrate.

A further particularly preferred example of an RNA substrate which can be cleaved by an oligomer of the "CUGANGA" type is human IL-1 receptor mRNA. The region which is sensitive to

cleavage is shown in SEQ ID NO. 15. An oligomer having the nucleotide sequence shown in SEQ ID NO. 16 can efficiently cleave such a substrate.

A further particularly preferred example of an RNA substrate which can be cleaved by an oligomer of the "CUGANGA" type is human angiotensinogen mRNA. The region which is sensitive to cleavage is shown in SEQ ID NO. 17. An oligomer having the nucleotide sequence shown in SEQ ID NO. 18 can efficiently cleave such a substrate.

The chimeric oligomers according to the invention have significant advantages compared to the structures of the state of the art. Thus the shortest ribozymes that have been previously used have a minimum length of 15+N+M nucleotides, the active centre being 15 nucleotides long and N and M being the length of the recognition sequences (Benseler et al., J. Am. Chem. Soc. 115 (1993), 8483-8484), and moreover they contain ribonucleotides in at least five positions of the catalytic centre (Paolella et al., EMBO J. 11 (1992), 1913-1919 and Yang et al., Biochemistry 31 (1992), 5005-5009).

In contrast the oligomers according to the invention only contain 4+N+M or 5+N+M nucleotides (in the case of the "GAAR" type) or 7+N+M or 8+N+M nucleotides (in the case of the "CUGANGA" type) in which N and M are preferably numbers in the range of 5-10. Furthermore they can contain a significantly smaller number of natural nucleotides without loss of activity. Due to the reduced length and reduced number of ribonucleotides there are significant advantages with regard to the synthesis of the molecules as well as with regard to the stability in vivo. Thus for example the chimeric oligomer described in example 2 of the present application of the "GAAR" type has a half-life in active 10 % human serum of several days. The in vivo stability can be additionally increased by a further reduction in the number of ribonucleotides.

In addition the oligomers according to the invention have a

very high in vivo activity since the RNA cleavage is promoted by protein factors that are present in the nucleus or cytoplasm of the cell. Examples of such protein factors which can increase the activity of hammerhead ribozymes are for example the nucleocapsid protein NCp7 of HIV1 (Müller et al., J. Mol. Biol. 242 (1994), 422-429) and the heterogeneous nuclear ribonucleoprotein A1 (Heidenreich et al., Nucleic Acids Res. 23 (1995), 2223-2228). Thus long RNA transcripts can be cleaved highly efficiently within the cell by the oligomers according to the invention.

Yet a further advantage of the chimeric oligomers according to the invention is the statistically rare occurrence of cleavage motifs of formulae (Va) or (Vb), or (VIa) or (VIb) (approximately one motif/5000 bp) This taken together with the individually selected recognition sequences results in the fact that statistically only the target RNA within the entire human RNA pool is cleaved by the respective chimeric oligomer, whereas only an unproductive binding but no cleavage occurs at other potential binding sites. In addition the oligomers according to the invention do <u>not</u> activate RNAse H due to their low content of deoxyribonucleotides and thus do not cause any unspecific cleavages.

A further surprising advantage compared to ribozymes of the state of the art is that in the structures according to the invention the RNA substrate forms a large part of the hammerhead structure by which means the dependence of the cleavage activity on the Mg^{2+} concentration is positively influenced.

Yet a further object of the present invention is a pharmaceutical composition that contains one or several chimeric oligomers as the active substance and optionally pharmaceutically acceptable auxiliary substances, additives and carriers. This pharmaceutical composition is excellently suitable for the production of an agent to specifically inactivate the expression of genes in eukaryotes, prokaryotes and viruses especially of human genes such as tumour genes or viral genes or RNA molecules

in a cell. Further areas of application are the inactivation of the expression of plant genes or insect genes. Thus the oligomers according to the invention can be used as a drug for humans and animals as well as a pesticide for plants.

For therapeutic applications the active substance is preferably administered at a concentration of 0.01 to 10,000 µg/kg body weight, particularly preferably of 0.1 to 1000 µg/kg body weight. The administration can for example be carried out by injection, inhalation (e.g. as an aerosol), as a spray, orally (e.g. as tablets, capsules, coated tablets etc.), topically or rectally (e.g. as suppositories).

The present invention provides a process for the specific inactivation of the expression of genes in which an active concentration of a chimeric oligomer is taken up into a cell (Lyngstadaas et al., EMBO J. 14 (1995) 5224-5229) so that the oligomer specifically cleaves a predetermined RNA molecule which is present in the cell, the cleavage preferably occurring catalytically. This process can be carried out in vitro on cell cultures as well as in vivo on living organisms (prokaryotes or eukaryotes such as humans, animals or plants).

Yet a further object of the present invention is the use of the chimeric oligomers as RNA restriction enzymes as well as a reagent kit for the restriction cleavage of RNA molecules which contains a chimeric oligomer and suitable buffer substances. In this case the oligomer and the buffer substances can be present in the form of solutions, suspensions or solids such as powders or lyophilisates. The reagents can be present together, separated from one another or optionally also on a suitable carrier. The oligomers according to the invention can also be used as a diagnostic agent or to identify the function of unknown genes.

This invention provides a method of alleviating psoriasis in a subject comprising administering to the subject an effective amount of the chimeric oligomers of this invention capable of cleaving RNAs encoding IL-2, or ICAM-1. This invention also

provides a method of alleviating common cold in a subject comprising administering to the subject an effective amount of the chimeric oligomers of this invention capable of cleaving RNAs encoding ICAM-1. This invention further provides a method of alleviating transplant rejection in a subject comprising administering to the subject an effective amount of the chimeric oligomers of this invention capable of cleaving RNAs encoding ICAM-1. Moreover, this invention provides a method of alleviating Kaposi's sarcoma in a subject comprising administering to the subject an effective amount of chimeric oligomers of this invention capable of cleaving RNAs encoding IL-6.

This invention also provides a method of treating human cancer in a subject comprising administering to the subject an effective amount of the chimeric oligomers of this invention capable of cleaving RNAs encoding PKC-α. The important role played by PKC-α in cancer is described in Dean et al. (Cancer Research 56 (1996), 3499-3507) and in McKay et al. (Nucleic Acids Research 24 (1996), 411-417) and in references contained therein.

Finally, this invention provides a method of treating hypertension in a human subject comprising administering to the subject an effective amount of the chimeric oligomers of this invention capable of cleaving RNAs encoding angiotensinogen.

All documents referred to in this application are incorporated in their entirety herein by reference.

The invention is additionally elucidated by the following figures and sequence protocols. They show:

Fig. 1 the schematic representation of a hammerhead structure and the corresponding nomenclature;

Fig. 2 a hammerhead structure with an oligomer of the "GAAR" type according to the invention;

Fig. 3 a hammerhead structure with an oligomer of the

"CUGANGA" type according to the invention;

Fig. 4a a hammerhead structure which is formed from an interleukin-2 mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 4b the reaction of the structures shown in Fig. 4a;

Fig. 5a a hammerhead structure which is formed from an ICAM-1 mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 5b the reaction of the structures shown in Fig. 5a;

Fig. 5c the reaction of the structures shown in Fig. 5a when the chimeric oligomer has 2'-allyloxy-nucleotide analogues in the flanking regions and U is replaced by 2'-allyloxy-thymidine;

Fig. 6a a hammerhead structure which is formed from an interleukin-6 mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 6b the reaction of the structures shown in Fig. 6a;

Fig. 6c the reaction of the structures shown in Fig. 6a whereby the substrate is 5'-fluorescently labelled and a variety of chimeric oligomers were tested with different analogues in the active centre "CUGAUGA";

Fig. 7a a hammerhead structure which is formed from an MDR-1 mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 7b the reaction of the structures shown in Fig. 7a;

Fig. 8a a hammerhead structure which is formed from a PKC-α mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 8b the reaction of the structures shown in Fig. 8a;

Fig. 9a a hammerhead structure which is formed from an interleukin-1α mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 9b the reaction of the structures shown in Fig. 9a;

Fig. 10a a hammerhead structure which is formed from a c-jun RNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 10b the reaction of the structures shown in Fig. 10a;

Fig. 11a a hammerhead structure which is formed from an interleukin-1 receptor mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 11b the reaction of the structures shown in Fig. 11a;

Fig. 12a a hammerhead structure which is formed from an angiotensinogen mRNA substrate and an oligomer according to the invention, wherein small letters denote ribonucleotides;

Fig. 12b the reaction of the structures shown in Fig. 12a;

SEQ ID NO. 1 shows the nucleotide sequence of the interleu-

kin-2 RNA substrate shown in Fig. 4a;

SEQ ID NO. 2 shows the sequence of the RNA-cleaving oligomer shown in Fig. 4a;

SEQ ID NO. 3 shows the nucleotide sequence of the ICAM-1 RNA substrate shown in Fig. 5a;

SEQ ID NO. 4 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 5a;

SEQ ID NO. 5 shows the nucleotide sequence of the interleukin-6 RNA substrate shown in Fig. 6a;

SEQ ID NO. 6 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 6a;

SEQ ID NO. 7 shows the nucleotide sequence of the MDR-1 RNA substrate shown in Fig. 7a;

SEQ ID NO. 8 shows the nucleotide sequence of the RNA-cleaving substrate shown in Fig. 7a;

SEQ ID NO. 9 shows the nucleotide sequence of the PKC-α RNA substrate shown in Fig. 8a;

SEQ ID NO. 10 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 8a;

SEQ ID NO. 11 shows the nucleotide sequence of the interleukin-1α RNA substrate shown in Fig. 9a;

SEQ ID NO. 12 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 9a;

SEQ ID NO. 13 shows the nucleotide sequence of the c-jun RNA substrate shown in Fig. 10a;

SEQ ID NO. 14 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 10a;

SEQ ID NO. 15 shows the nucleotide sequence of the interleukin-1 receptor RNA substrate shown in Fig. 11a;

SEQ ID NO. 16 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 11a;

SEQ ID NO. 17 shows the nucleotide sequence of the angiotensinogen RNA substrate shown in Fig. 12a and

SEQ ID NO. 18 shows the nucleotide sequence of the RNA-cleaving oligomer shown in Fig. 12a.

In the sequences stated in SEQ ID NO. 1-18 the T at the end of the nucleotide is in each case coupled via its 3' position to the 3' position of the penultimate nucleotide (iT).

The invention is elucidated further by the following examples.

Example 1

Cleavage of an interleukin-2 RNA substrate

An interleukin-2 RNA substrate having the nucleotide sequence shown in SEQ ID NO. 1 was produced by chemical solid phase synthesis. Furthermore a chimeric oligomer was synthesized having the nucleotide sequence shown in SEQ ID NO. 2. Synthesis and removal of protecting groups was carried out in the case of the RNA substrate as well as of the chimeric oligomer by means of solid phase techniques (Wincott et al., Nucleic Acids Res. 23 (1995), 2677-2684). Purification was by means of anion-exchange HPLC (Sproat et al., Nucleosides and Nucleotides 14 (1995), 255-273). It was synthesized on an inverse thymidine support (Ortigao et al., Antisense Research and Development 2 (1992), 129-146) based either on aminopropyl-modified controlled pore

glass or preferably aminomethylpolystyrene (McCollum and Andrus, Tetrahedron Letters 32 (1991), 4069-4072). Suitable protected 2'-methoxynucleoside-3'-O-phosphoramiditeswere synthesized as described by Sproat and Lamond in Oligonucleotides and Analogs - A practical approach, F. Eckstein, publ. (IRL-Press, Oxford, UK, (1991), 49-86). The 2'-allyloxy-nucleotidemonomers for the solid phase synthesis were also synthesized by standard procedures (Sproat in: Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs - Synthesis and Properties, S. Agrawal, publ./ Humana Press, Totowa, New Jersey, (1993), 115-141)).

The sequences of the chimeric oligomer outside of the active centre are composed of nucleotide analogue building blocks which are modified with a methoxy group at the 2'-C atom of the ribose. In the active centre "GAAA" of the oligonucleotide G^{12} at position 1 is a ribonucleotide, the residues A^{13} and A^{14} at positions 2 and 3 are 2'-methoxy-modifiednucleotides and $A^{15.1}$ at position 4 is a ribonucleotide.

400 pmol interleukin-2 substrate was reacted with 400 pmol of the oligomer in the presence of 10 mmol/l $MgCl_2$, 50 mmol/l Tris HCl, 1 mmol/l EDTA at pH 7.5 and 37°C in a reaction volume of 100 µl. Aliquots were taken at certain times and admixed with excess EDTA to prevent further cleavage. The oligomer mixture present at the different times was then separated by electrophoresis on a 20 % polyacrylamide gel in the presence of 7 M urea and made visible by staining with "stains-all".

The result of this reaction is shown in Fig. 4b. This shows that the band (40-mer) allocated to the substrate disappears as the incubation period increases and the intensity of the bands of the cleavage products (31-mer and 9-mer) increases.

Similar results to those shown in Fig. 4b were obtained with an oligomer that contains a deoxyguanosine at position G^{12} instead of a guanosine. The following two sequences of the active cen-

tre Z also led to an effective cleavage of the 40-mer RNA substrate but with a slightly lower cleavage rate:

(i) position G^{12}: guanosine;
positions A^{13} and A^{14}: 2'-O-methyladenosine;
position $A^{15.1}$: 2'-O-(2-hydroxyethyl)-adenosine;
(ii) position G^{12}: guanosine;
positions A^{13}, A^{14} and $A^{15.1}$: 2'-O-(2-hydroxyethyl)-adenosine.

Example 2

Cleavage of an ICAM-1 RNA substrate

An ICAM-1 RNA substrate having the nucleotide sequence shown in SEQ ID NO. 3 and an RNA-cleaving oligomer having the nucleotide sequence shown in SEQ ID NO. 4 were synthesized in an analogous manner to that described in example 1. The nucleotide building blocks used for the oligomer were also the same as those described in example 1 except that A^{13} and A^{14} were ribonucleotides.

The result of the reaction is shown in Fig. 5b. The reaction conditions were as described in example 1 except that 1 nmol substrate and 100 pmol chimeric oligomer were used. It can be seen that the band of the substrate (43-mer) disappears as the incubation period increases while the intensity of the bands of the products (34-mer and 9-mer) increases.

A chimeric oligomer was synthesized in an analogous manner which contained four ribonucleotides in the active centre Z and 2'-allyloxy-nucleotides in the flanking regions X and Y. 2'-Allyloxythymidine was used instead of 2'-O-allyl-uridine at the positions in X and Y labelled U. The results of the cleavage of the 43-mer RNA substrate by this oligomer are shown in Fig. 5c.

Oligomers based on 2'-O-allyloxy-nucleotides are strongly preferred over oligomers based on 2'-methoxy-nucleotides (Iribar-

ren et al., Proc. Natl. Acad. Sci. USA 87 (1990), 7747-7751) especially for therapeutic applications due to their greatly reduced unspecific binding.

Example 3

Cleavage of an interleukin-6 RNA substrate

An interleukin-6 RNA substrate having the nucleotide sequence shown in SEQ ID NO. 5 and a chimeric oligomer having the nucleotide sequence shown in SEQ ID NO. 6 were synthesized in an analogous manner to that described in example 1. 2'-Methoxy-modified nucleotides were used in sequences outside the active centre to produce the oligomer. Ribonucleotides were used for the active centre "CUGAUGA".

The reaction was carried out as described in example 1. The result is shown in Fig. 6b. The intensity of the bands of the substrate (32-mer) decreases as the incubation period increases and the intensity of the bands of the cleavage products (23-mer and 9-mer) increases.

Further reactions were performed as follows: A 5'-fluorescein labelled substrate having the nucleotide sequence shown in SEQ ID NO. 5 was synthesized using a commercially available fluorescein phosphoramidite monomer, and subjected to cleavage reactions as described above, however using a variety of chimeric oligomers each having the nucleotide sequence shown in SEQ ID NO. 6, all bearing 2'-allyloxy modified nucleotides in regions X and Y but different analogues in the active centre "CUGANGA". The reactions were carried out as described in example 1, except that the chimeric oligomer concentration was 10 µmol/l and the initial substrate concentration was 2 µmol/l. The time course of the reaction is shown in Fig. 6c using fluorescence detection, so only the full length fluorescein labelled 32mer substrate and the 5'-fluorescein labelled 23mer product 1 are detected. The lanes comprised the following:

Lane 1: Substrate plus chimeric oligomer with 2'-C-allyl-2'-deoxycytidine at position C^3, 2'-amino-2'-deoxyuridine at positions U^4 and U^7 and ribonucleotides at positions G^5, A^6, G^8, and A^9 in the active centre "CUGANGA".

Lane 2: Substrate plus chimeric oligomer with 2'-C-allyl-2'-deoxycytidine at position C^3, 2'-C-allyl-2'-deoxyuridine at positions U^4 and U^7 and ribonucleotides at positions G^5, A^6, G^8, and A^9 in the active centre "CUGANGA".

Lane 3: Substrate plus chimeric oligomer with 2'-allyloxy-2'-deoxycytidine at position C^3, 2'-C-allyl-2'-deoxyuridine at positions U^4 and U^7 and ribonucleotides at positions G^5, A^6, G^8, and A^9 in the active centre "CUGANGA".

Lane 4: Substrate plus chimeric oligomer with 2'-allyloxy-2'-deoxycytidine at position C^3, 2'-amino-2'-deoxyuridine at positions U^4 and U^7 and ribonucleotides at positions G^5, A^6, G^8, and A^9 in the active centre "CUGANGA".

Lane 5: Substrate plus chimeric oligomer containing all ribonucleotides in the active centre "CUGANGA".

Lane 6: Substrate only.

The chimeric oligomer used in lane 5 is clearly the most efficient, however, since it contains pyrimidine ribonucleotides in the active centre "CUGANGA" it is less suitable for in vivo use. The chimeric oligomers used in lanes 1 and 4 are somewhat less active, however they contain no pyrimidine ribonucleotides and are thus very well suitable for cell culture and in vivo work.

Example 4

Cleavage of a MDR-1 RNA substrate

A MDR-1 RNA substrate having the nucleotide sequence shown in SEQ ID NO. 7 and an RNA-cleaving oligomer having the nucleotide sequence shown in SEQ ID NO. 8 were synthesized in an analogous manner to that described in example 1. The oligomer was synthesized using the nucleotide building blocks stated in example 3.

The reaction was carried out as described in example 1.
The result is shown in Fig. 7b.

Example 5

Synthesis, removal of protecting groups and purification of a chimeric oligomer of the "GAAA" type for cleaving an RNA motif on human ICAM-1 RNA

The chimeric oligomer 5'-CCCCT*CgaaaT*CAT*GT*C-iT, in which capital letters denote 2'-allyloxy-nucleotides (T* is 2'-allyloxythymidine), small letters denote ribonucleotides and -iT denotes a 3'-3'-phosphodiester bond to deoxythymidine, was synthesized according to the "trityl-off" technique by a solid phase-ß-cyanoethyl phosphoroamidite technique (Sinha et al., Nucleic Acids Res. 12 (1984), 4539-4557) on a 1 µmol scale using an inverse thymidine support (Ortigao et al., Supra), protected 2'-allyloxy-nucleotide monomers (Sproat in Methods in Molecular Biology, Supra) and commercially available 2'-O-tert.-butyldimethylsilyl-protected ribonucleotide monomers using a longer coupling period of 15 min. At the end of the synthesis the oligomer was cleaved in the synthesis apparatus from the controlled pore glass or polystyrene suppcrt during a period of 2 h using 2 ml 33 % aqueous ammonia/ethanol (3:1 v/v). The solution was then kept at 60°C for 6-8 h in a sealed reaction vessel in order to remove all base-labile protecting groups. The reaction vessel was cooled and the solution was evaporated to dryness.

The residue was taken up in 300 µl ethanol/water (1:1 v/v) and transferred to a 1.5 ml reaction vessel. Subsequently the solution was again lyophilized.

Silyl protecting groups were removed by resuspending the residue in 200 µl dry triethylamine/triethyl-amine· 3 HF/N-methyl-2-pyrrolidinone (3:4:6 v/v/v) and incubating for 2 h at 60°C (Wincott et al., Supra). The chimeric oligomer which had been completely freed of protecting groups was then precipitated by addition of 20 µl 3 M aqueous sterile sodium acetate and subsequently of 1 ml 1-butanol. The reaction vessel was kept at -20°C for 15 minutes and the precipitate was collected by centrifugation for 10 min. The supernatant was carefully removed by decanting, the pellet was taken up in 300 µl 80 % ethanol and the resulting solution was lyophilized.

The crude product was firstly analyzed and then purified for 30 min in four aliquots by anion-exchange HPLC on a Dionex Nucleopak PA-100 column (9 x 250 mm) at 55°C using a lithium perchlorate gradient of 70-230 mM in water/acetonitrile (9:1 v/v) containing 25 mM Tris pH 7.0. The main peak was collected and the chimeric oligomer was precipitated by addition of 4 volumes of 1-propanol or 2-propanol (Sproat et al., (1995) Supra). The precipitate was collected by centrifugation, washed and then dried in a vacuum. The yield of purified product was 2.8 mg.

Example 6

Cleavage of a PKC-α RNA substrate

A PKC-α RNA substrate having the nucleotide sequence shown in SEQ ID NO. 9 and an RNA-cleaving oligomer having the nucleotide sequence shown in SEQ ID N0. 10 were synthesized in an analogous manner to that described in example 1. The oligomer was synthesized with 2'-allyloxy-modified nucleotides in the sequences outside the active centre, and with ribonucleotides in the active centre "GAAA".

The reaction was carried out as described in example 1, except that the chimeric oligomer concentration was 10 µmol/l and the RNA substrate concentration was 2 µmol/l. The result is shown in Fig. 8b.

Example 7

Cleavage of an IL-1α RNA substrate

An IL-1α RNA substrate having the nucleotide sequence shown in SEQ ID NO. 11 and an RNA-cleaving oligomer having the nucleotide shown in SEQ ID NO. 12 were synthesized in an analogous manner to that described in example 1. The oligomer was synthesized with 2'-methoxy-modified nucleotides in the sequences outside the active centre, and with ribonucleotides in the active centre "GAAA".

The reaction was carried out as described in example 1, except that the chimeric oligomer concentration was 10 µmol/l and the RNA substrate concentration was 2 µmol/l. The result is shown in Fig. 9b.

Example 8

Cleavage of a c-jun RNA substrate

A c-jun RNA substrate having the nucleotide sequence shown in SEQ ID NO. 13 and an RNA-cleaving oligomer having the nucleotide sequence shown in SEQ ID NO. 14 were synthesized in an analogous manner to that described in example 1. The oligomer was synthesized with 2'-methoxy-modified nucleotides in the sequences outside the active centre, and with ribonucleotides in the active centre "CUGAUGA".

The reaction was carried out as described in example 1, except that the chimeric oligomer concentration was 10 µmol/l and the RNA substrate concentration was 2 µmol/l. The result is shown in Fig. 10b.

Example 9

Cleavage of an IL-1 receptor RNA substrate

An IL-1 receptor RNA substrate having the nucleotide sequence shown in SEQ ID NO. 15 and an RNA-cleaving oligomer having the nucleotide sequence shown in SEQ ID NO. 16 were synthesized in analogous manner to that described in example 1. The oligomer was synthesized with 2'-allyloxy-modified nucleotides in the sequences outside the active centre, and with ribonucleotides in the active centre "CUGAUGA".

The reaction was carried out as described in example 1, except that the chimeric oligomer concentration was 10 µmol/l and the RNA substrate concentration was 2 µmol/l. The result is shown in Fig. 11b.

Example 10

Cleavage of an angiotensinogen RNA substrate

An angiotensinogen RNA substrate having the nucleotide sequence shown in SEQ ID N0. 17 and an RNA-cleaving oligomer having the nucleotide sequence shown in SEQ ID N0. 18 were synthesized in an analogous manner to that described in example 1. The oligomer was synthesized with 2'-methoxy-modified nucleotides in the sequences outside the active centre, and with ribonucleotides in the active centre "CUGAUGA".

The reaction was carried out as described in example 1, except that the chimeric oligomer concentration was 10 µmol/l and the RNA substrate concentration was 2 µmol/l. The result is shown in Fig. 12b.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: VimRx Holdings, Ltd.
(B) STREET: 2751 Centerville Road, Suite 210
(C) CITY: Little Falls II, Wilmington
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): Delaware 19808

(ii) TITLE OF INVENTION: Chimeric oligomers having an RNA-cleavage activity

(iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: DE 195 42 404.2
(B) FILING DATE: 14-NOV-1995

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AACAACAUUC AUGUGUGAAU AUGCUGAUGA GACAGCAACT 40

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GUUGCUGUCG AAAUGUUGUU T 21

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACAUGAUUG AUGGAUGUUA AAGUCUAGCC UGAUGAGAGG GGT 43

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCCCUCGAAA UCAUGUCT 18

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

UACCACUUGA AACAUUUUAU GUAUUAGUUU UT 32

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AAAACUAACU GAUGAAAGUG GUAT 24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCAGCUUAUG AAAUCUUCAA GAUAAUUGAU T 31

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AUCAAUCUGA UGAAUAAGCU GCT 23

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAUCCCUUUC CUUUGGAGUU UCGGAGCUGA UGAAGAUGCC T 41

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGCAUCUGAA AAAGGGAUT 19

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

UGUUUGCCUU CUACUUUUAA GUUGCUGAUG AACUCUUAT 39

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

UAAGAGUGAA AGGCAAACT 19

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

UAGGUGGAGU UGAAAGAGUU AAGAAUGCUC GAUAAAAUCT 40

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

UUUUAUCCUG AUGAAACUCC AT 22

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AAGAAGAAUA UGAAAGUGUU ACUCAGACUU AUUT 34

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

UAAGUCUCUG AUGAAUAUUC UT 22

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AAUACGUGAA AGAUGCAAGC ACCUGAAUUU CUGUUUGAT 39

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

UCAAACACUG AUGAACGUAU UT 22

Patent Claims

1. Chimeric oligomer having an RNA-cleavage activity which has a structure according to formula (I):

$$5'\text{-}X\text{-}Z\text{-}Y\text{-}3' \qquad (I)$$

in which X and Y represent oligomeric sequences containing building blocks composed of unmodified nucleotides or/and nucleotide analogues and contribute to the formation of a specific hybridisation of the oligomer with an RNA-substrate,

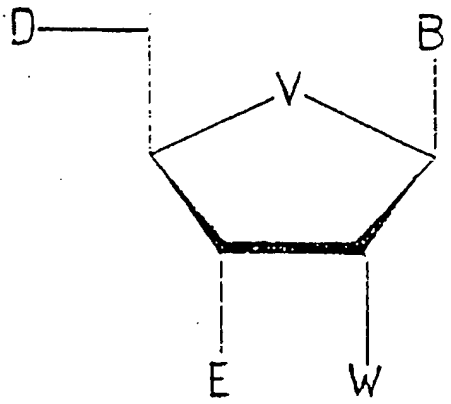
in which X and Y each contain at least one building block that differs from deoxyribonucleotides and ribonucleotides,

wherein Z represents the active centre of the chimeric oligomer having a structure of formulae (IIIa), (IIIb), (IVa) or (IVb):

$5'\text{-}G^{12}A^{13}A^{14}R^{15.1}\text{-}3'$ (IIIa)
$5'\text{-}N^{\lambda 12}G^{12}A^{13}A^{14}R^{15.1}\text{-}3'$ (IIIb)
$5'\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}\text{-}3'$ (IVa)
$5'\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}N^{9A}\text{-}3'$ (IVb)

in which the building blocks of the structures (IIIa), (IIIb), (IVa), (IVb) are selected from unmodified nucleotides or/and nucleotide analogues,

in which the building blocks have a structure according to formula (II):



(II)

in which B denotes a residue, which is a naturally occurring nucleobase or a functional equivalent thereof, which is in particular selected from the group comprising adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), uracil-5-yl (ψ) hypoxanthin-9-yl (I), thymin-1-yl (T), 5-methyl- cytosin-1-yl (5MeC), 2,6-diaminopurin-9-yl (AminoA), purin-9-yl (P), 7-deazaadenin-9-yl (c^7A), 7-deazaguanin-9-yl (c^7G), 5-propynylcytosin-1-yl (5-pC), 5-propynyluracil-1-yl (5-pU), isoguanin-9-yl, 2-aminopurin-9-yl, 6-methyluracil-1-yl, 4-thiouracil-1-yl, 2-pyrimidone-1-yl and quinazoline-2,4-dione-1-yl;

V independently denotes an O, S, NH or CH_2-group,

W independently denotes an H or OH or a straight-chained or branched alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy with 1 to 10 C-atoms, which can be optionally substituted by one or several halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxyl or/and mercapto groups, or is selected from -COOH, $-CONH_2$, $CONHR^1$, $CONR^1R^2$, $-NH_2$, $-NHR^1$, $-NR^1R^2$, $-NHCOR^1$, -SH, SR^1, -F, $-ONH_2$, $-ONHR^1$, $-ONR^1R^2$, NHOH, $-NHOR^1$, $-NR^2OH$ and $-NR^2OR^1$, in which R^1 and R^2 independently denote unsubstituted or substituted alkyl, alkenyl or alkynyl groups as defined above,

D and E denote residues which together form a phosphodiester or phosphorothioate diester bond between adjacent nucleosides or analogues or together form an analogue of an internucleosidic bond,

in which the structures (IIIa) or (IIIb) do not contain a total of more than two deoxyribonucleotides and the structures (IVa) or (IVb) do not contain a total of more than four deoxyribonucleotides and

in which G in each case independently denotes a building block according to formula (II), in which B is guanin-9-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from I, c^7G and isoguanin-9-yl;

A in each case independently denotes a building block according to formula (II), in which B is adenin-9-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from aminoA, P, c^7A and 2-aminopurin-9-yl;

U in each case independently denotes a building block according to formula (II), in which B is uracil-1-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from ψ, T, 5-pU, 6-methyluracil-1-yl, 4-thiouracil-1-yl, 2-pyrimidone-1-yl and quinazoline-2,4-dione-1-yl;

C in each case independently denotes a building block according to formula (II), in which B is cytosin-1-yl or a functional equivalent thereof for that particular location in the hammerhead core structure, which is preferably selected from 5MeC, 5-pC

and 2-pyrimidone-1-yl;

R in each case independently denotes a building block according to formula (II), in which B independently denotes G or A as defined above;

N in each case independently denotes a building block according to formula (II).

2. Oligomer as claimed in claim 1, wherein the regions X and Y do not contain any ribonucleotides at pyrimidine positions.

3. Oligomer as claimed in claim 1 or 2, wherein the regions X and Y do not contain any ribonucleotides.

4. Oligomer as claimed in one of the claims 1-3, wherein the regions X and Y are essentially composed of building blocks having the structure (II), in which W is in each case independently selected from optionally substituted alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy residues with 1 to 10 C atoms.

5. Oligomer as claimed in one of the claims 1-4, wherein the regions X and Y each independently contain 3 to 40 building blocks.

6. Oligomer as claimed in one of the claims 1-5, wherein the active centre Z contains one or several building blocks having the structure (II), in which W in each case is independently selected from optionally substituted alkyl,

alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy residues with 1 to 5 C atoms.

7. Oligomer as claimed in one of the claims 1-6, wherein the building blocks of the active centre Z are linked by phosphodiester bonds.

8. Oligomer as claimed in one of the claims 1-7, wherein the 3′ end is protected against exonuclease degradation.

9. Oligomer as claimed in one of the claims 1-8, wherein the active centre Z has the structure (IIIa) or (IIIb) and has cleavage activity for an RNA-substrate that contains a structure of formulae (Va) or (Vb):

$5'\text{-}U^{16.1}H^{17}\text{-}S\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}R^{10.1}\text{-}3'$ (Va)

$5'\text{-}U^{16.1}H^{17}\text{-}S\text{-}C^{3}U^{4}G^{5}A^{6}N^{7}G^{8}A^{9}N^{9A}R^{10.1}\text{-}3'$ (Vb)

in which N, H, R, S, G, A, U and C represent ribonucleotides and
N denotes G, A, U or C;
H denotes A, C or U;
R denotes A or G and
S denotes an RNA-sequence capable of forming a hairpin structure.

10. Oligomer as claimed in claim 9, wherein Z has the structure (IIIa) or (IIIb) and contains no more than two ribonucleotides.

11. Oligomer as claimed in claim 10, wherein Z has the structure (IIIa) or (IIIb) and contains no more

than one ribonucleotide.

12. Oligomer as claimed in claim 11, wherein Z has the structure (IIIa) or (IIIb) and contains no ribonucleotides and no deoxyribonucleotides.

13. Oligomer as claimed in one of the claims 9-12, wherein G^{12} denotes a ribonucleotide, a deoxyribonucleotide or a building block, in which W is a C_1-C_4-alkyl, alkenyl, alkoxy, or alkenyloxy group optionally substituted with OH.

14. Oligomer as claimed in claim 13, wherein W is a (2-hydroxyethoxy) residue.

15. Oligomer as claimed in one of the claims 9-14, wherein A^{13}, A^{14} or/and $R^{15.1}$ are selected from building blocks, in which W is preferably a C_1-C_4-alkyl, alkenyl, alkoxy, or alkenyloxy group, optionally substituted with OH.

16. Oligomer as claimed in claim 15, wherein W is selected from methoxy, (2-hydroxyethoxy) and allyloxy residues.

17. Oligomer as claimed in one of the claims 9-16, wherein the RNA-substrate is selected from the group comprising human interleukin-2 mRNA, human ICAM-1 mRNA, human TGF-ß mRNA, human tissue factor pre-mRNA, human protein kinase C-α mRNA, human factor KBF-1 mRNA, human 5-lipoxygenase mRNA, human interleukin-8 RNA, human interleukin-5 RNA, human interleukin-1 receptor mRNA, human interleukin-1-α mRNA, human 12-lipoxygenase mRNA, the transcript of the human

cytomegalovirus DNA-polymerase gene and a transcript of the human papilloma virus type 8.

18. Oligomer as claimed in one of the claims 9-17, wherein the RNA-substrate contains a region from human interleukin-2 mRNA having the nucleotide sequence shown in SEQ ID No. 1.

19. Oligomer as claimed in claim 18, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 2.

20. Oligomer as claimed in one of the claims 9-17, wherein the RNA-substrate contains a region from human ICAM-1 mRNA having the nucleotide sequence shown in SEQ ID No. 3.

21. Oligomer as claimed in claim 20, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 4.

22. Oligomer as claimed in one of the claims 9-17, wherein the RNA-substrate contains a region from human PKC-α RNA having the nucleotide sequence shown in SEQ ID No. 9.

23. Oligomer as claimed in claim 22, wherein the oligomer contains the sequence shown in SEQ ID No. 10.

24. Oligomer as claimed in one of the claims 9-17, wherein the RNA substrate contains a region from human interleukin-

1α mRNA having the nucleotide sequence shown in SEQ ID No. 11.

25. Oligomer as claimed in claim 24, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 12.

26. Oligomer as claimed in one of the claims 1-8, wherein the catalytic centre Z has the structure (IVa) or (IVb) and exhibits cleavage activity for an RNA-substrate that contains a structure of formulae (VIa) or (VIb):

$5'\text{-}Y^{11.1}G^{12}A^{13}A^{14}R^{15.1}\text{-}S\text{-}U^{16.1}H^{17}\text{-}3'1$ (VIa)

$5'\text{-}Y^{11.1}N^{\lambda 12}G^{12}A^{13}A^{14}R^{15.1}\text{-}S\text{-}U^{16.1}H^{17}\text{-}3'$ (VIb)

in which N, H, Y, R, S, G, A, U and C represent ribonucleotides and
N denotes G, A, U or C,
H denotes A, C or U,
R denotes A or G
Y denotes C or U and
S is an RNA sequence capable of forming a hairpin structure.

27. Oligomer as claimed in claim 26, wherein Z has the structure (IVa) or (IVb) and contains no more than six ribonucleotides.

28. Oligomer as claimed in claim 26 or 27, wherein Z contains no pyrimidine ribonucleotides.

29. Oligomer as claimed in one of the claims 26-28, wherein

one or several building blocks at the positions G^5, A^6, N^7, G^8 and A^9 are ribonucleotides, in which N^7 is a purine base, in particular A.

30. Oligomer as claimed in one of the claims 26-29, wherein
C^3, U^4 and $N^{\lambda 12}$ are selected from building blocks, in which W is an optionally OH-substituted C_1-C_4 alkyl, alkenyl, alkoxy or alkenyloxy group or an NH_2 group.

31. Oligomer as claimed in claim 30, wherein
W is selected from methoxy, (2-hydroxyethyloxy), allyloxy, allyl and NH_2 residues.

32. Oligomer as claimed in one of the claims 26-31, wherein
the RNA-substrate is selected from the group comprising human interleukin-6 mRNA, human MDR-1 mRNA, human monocyte chemotactic protein RNA, human macrophage scavenger receptor type II mRNA, human macrophage scavenger receptor type I mRNA, human macrophage-inflammatory protein-1α mRNA, human p53 RNA, human jun-B mRNA, human c-jun RNA, human interferon-γ type II mRNA, human hepatocyte growth factor mRNA, human HER2 mRNA, human Alzheimer's disease amyloid mRNA, human interleukin-1 mRNA, human interleukin-1 receptor mRNA, human 3-hydroxy-3-methylglutaryl coenzyme A reductase RNA, human angiotensinogen mRNA, human angiotensin-converting enzyme mRNA, human acyl-coenzyme A: cholesterol acyltransferase mRNA, human PDGF receptor mRNA, human TNF receptor mRNA, human TGF ß mRNA, human NF-kappa B p65 subunit mRNA, human c-myc RNA, human 12-lipoxygenase mRNA, human interleukin-4 RNA, human interleukin-10 mRNA, human basic FGF mRNA, human EGF-receptor mRNA, human c-myb mRNA, human c-fos RNA, human bcl-2 mRNA, human bcl-1 mRNA, human ICAM-1 mRNA, a transcript of human papilloma virus type 11 and transcripts of human papilloma virus type 16

and type 18.

33. Oligomer as claimed in one of the claims 26-32, wherein the RNA-substrate contains a region from human interleukin-6 mRNA having the nucleotide sequence shown in SEQ ID No. 5.

34. Oligomer as claimed in claim 33, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 6.

35. Oligomer as claimed in one of the claims 26-32, wherein the RNA substrate contains a region from human MDR-1 mRNA having the nucleotide sequence shown in SEQ ID No. 7.

36. Oligomer as claimed in claim 35, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 8.

37. Oligomer as claimed in one of the claims 26-32, wherein the RNA substrate contains a region from c-jun RNA having the nucleotide sequence shown in SEQ ID No. 13.

38. Oligomer as claimed in claim 37, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 14.

39. Oligomer as claimed in one of the claims 26-32, wherein the RNA substrate contains a region from human interleukin-

1 receptor mRNA having the nucleotide sequence shown in SEQ ID No. 15.

40. Oligomer as claimed in claim 39, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 16.

41. Oligomer as claimed in one of the claims 26-32, wherein the RNA substrate contains a region from human angiotensinogen RNA having the nucleotide sequence shown in SEQ ID No. 17.

42. Oligomer as claimed in claim 41, wherein the oligomer contains the nucleotide sequence shown in SEQ ID No. 18.

43. Oligomer as claimed in one of the claims 1-42, wherein it is linked to a prosthetic group.

44. Pharmaceutical composition that contains one or several chimeric oligomers as claimed in one of the claims 1-43 as the active substance and optionally pharmaceutically acceptable auxiliary substances, additives and carriers.

45. Use of a chimeric oligomer as claimed in one of the claims 1-43 or a pharmaceutical composition as claimed in claim 44 to produce an agent for the specific inactivation of the expression of genes in eukaryotes, prokaryotes and viruses.

46. Use as claimed in claim 45 for the specific inactivation of the expression of human genes in a cell.

47. Use as claimed in claim 46 for the specific inactivation of the expression of tumor genes.

48. Use as claimed in claim 45 for the specific inactivation of the expression of viral genes or RNA molecules in a cell.

49. Use as claimed in claim 45 for the specific inactivation of the expression of plant genes.

50. Use as claimed in one of the claims 45-49,
wherein
the active substance is administered at a concentration of 0.01 to 10,000 µg/kg body weight.

51. Use as claimed in one of the claims 45-50,
wherein
the active substance is administered by injection, inhalation, as a spray, orally, topically or rectally.

52. Process for the specific inactivation of the expression of genes,
wherein
a chimeric oligomer as claimed in one of the claims 1-43 is introduced into a cell in an active concentration so that the oligomer specifically cleaves a predetermined RNA molecule present in the cell.

53. Process as claimed in claim 52,
wherein
the cleavage occurs catalytically.

54. Use of a chimeric oligomer as claimed in one of the claims 1-43 as an RNA restriction enzyme.

55. A kit for the restriction cleavage of RNA molecules,
wherein
it contains a chimeric oligomer as claimed in one of the

claims 1-43 and suitable buffer substances.

56. Use of a chimeric oligomer as claimed in one of the claims 1-43 as a diagnostic agent.

57. Use of a chimeric oligomer as claimed in one of the claims 1-43 to identify the function of unknown genes.

58. A method of alleviating psoriasis in a subject comprising administering to the subject an effective amount of an oligomer selected from the group consisting of the oligomers of claims 1-16 and 26-31, wherein the RNA substrate is an IL-2 or ICAM-1 RNA.

59. A method of alleviating common cold in a subject comprising administering to the subject an effective amount of an oligomer selected from the group consisting of the oligomers of claims 1-16 and 26-31, wherein the RNA substrate is an ICAM-1 RNA.

60. A method of alleviating transplant rejection in a subject comprising administering to the subject an effective amount of an oligomer selected from the group consisting of the oligomers of claims 1-16 and 26-31, wherein the RNA substrate is an ICAM-1 RNA.

61. A method of alleviating Kaposi's sarcoma in a subject comprising administering to the subject an effective amount of an oligomer selected from the group consisting of the oligomers of claims 1-16 and 26-31, wherein the RNA substrate is an IL-6 RNA.

62. A method of treating cancer in a subject comprising administering to the subject an effective amount of an oligomer selected from the group consisting of the oligomers of claims 1-16 and 26-31, wherein the RNA substrate is a PKC-α RNA.

63. A method of treating hypertension in a subject comprising administering to the subject an effective amount of an oligomer selected from the group consisting of the oligomers of claims 1-16 and 26-31, wherein the RNA substrate is an angiotensinogen RNA.

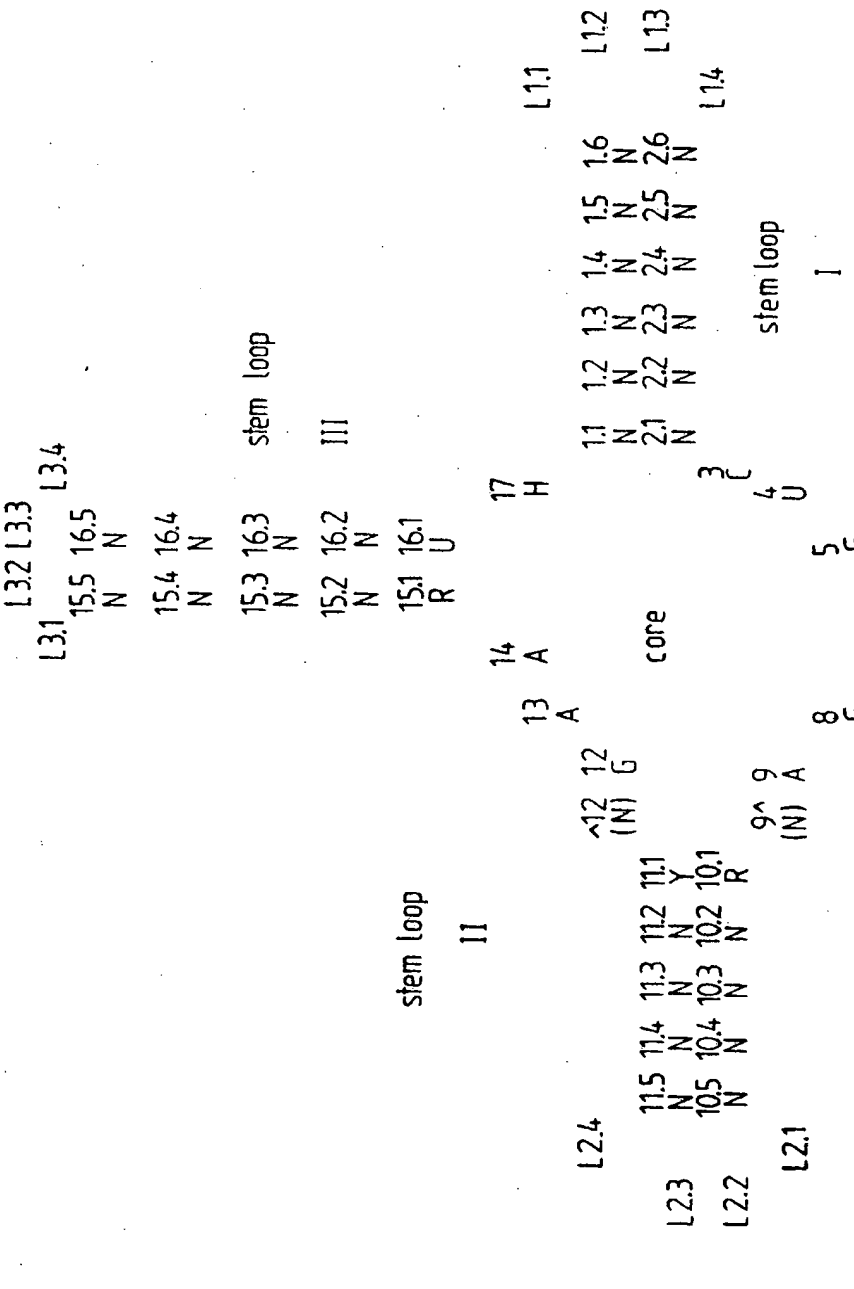


Fig.1 Numbering system for the hammerhead ribozyme according to Hertel et al. (Nucl.Acids Res.20,3252(1992).)
(Positions ^12 and 9^ are defined in the text)

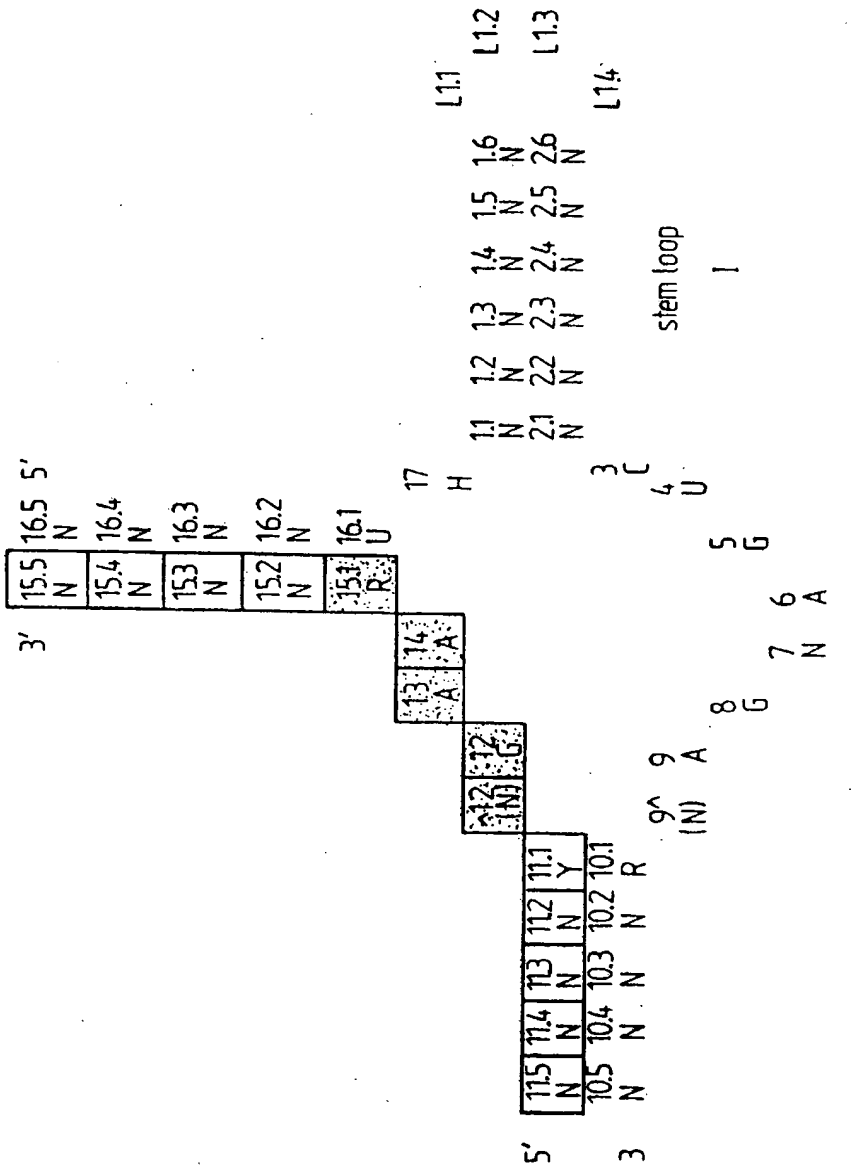


Fig. 2 Chimeric oligonucleotide of the "GAAA" type
Shaded areas represent the active cente (Z), unshaded areas represent the recognition sequences (X,Y), the cleavage site is the interribonucleotide bond after H17.

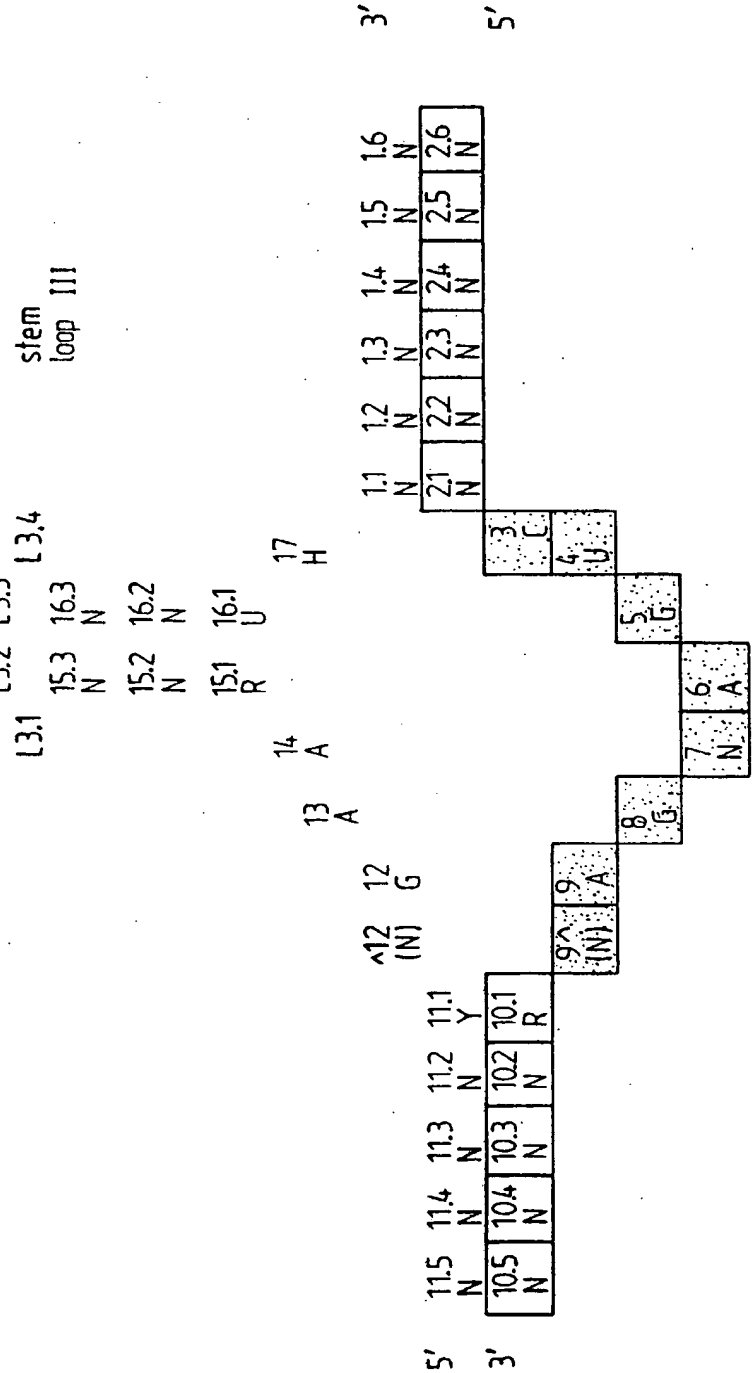


Fig. 3 Chimeric oligonucleotide of the "CUGANGA" type
Shaded areas represent the active cente (Z), unshaded areas represent the recognition sequences (X,Y), the cleavage site is the interribonucleotide bond after H17.

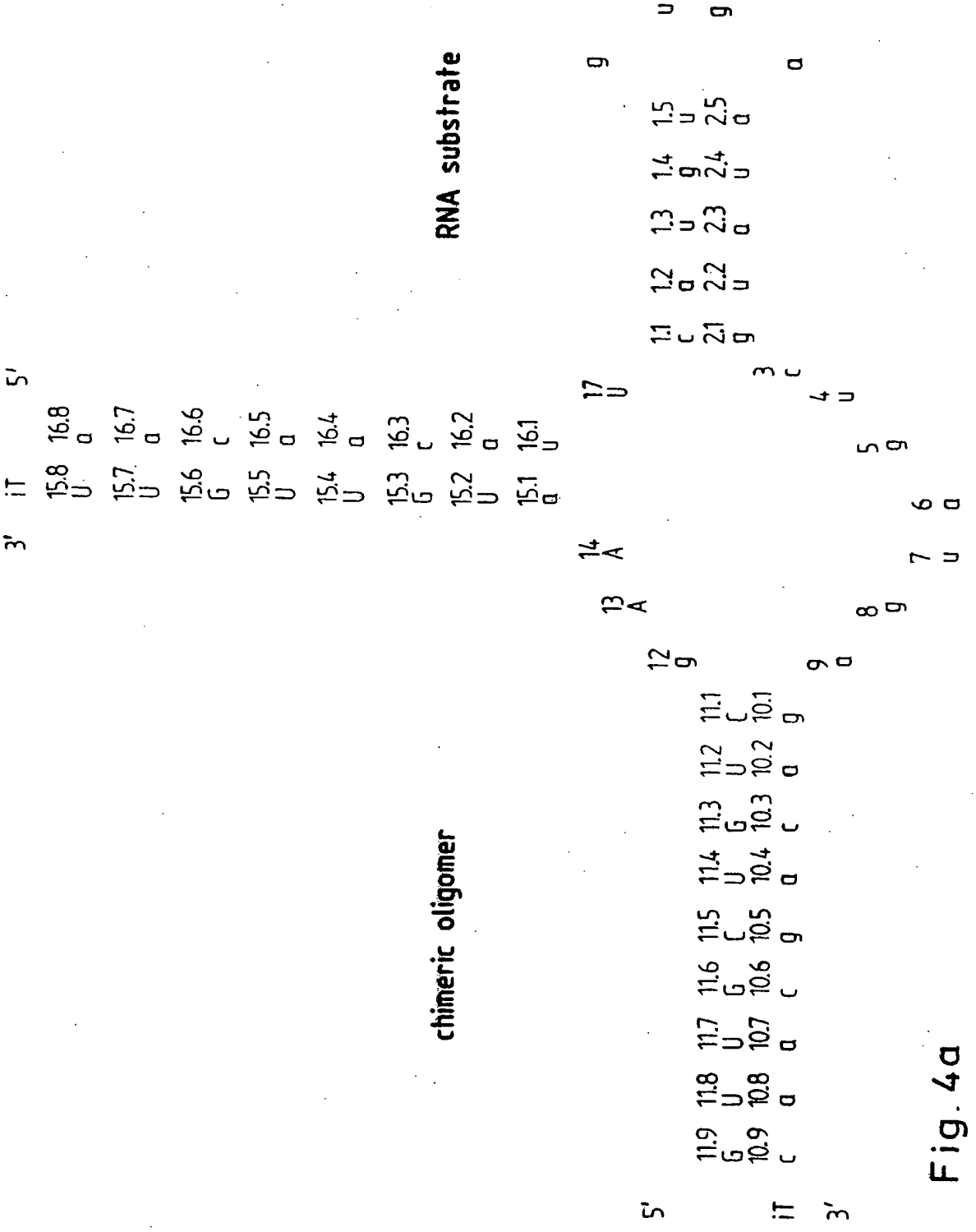


Fig. 4a

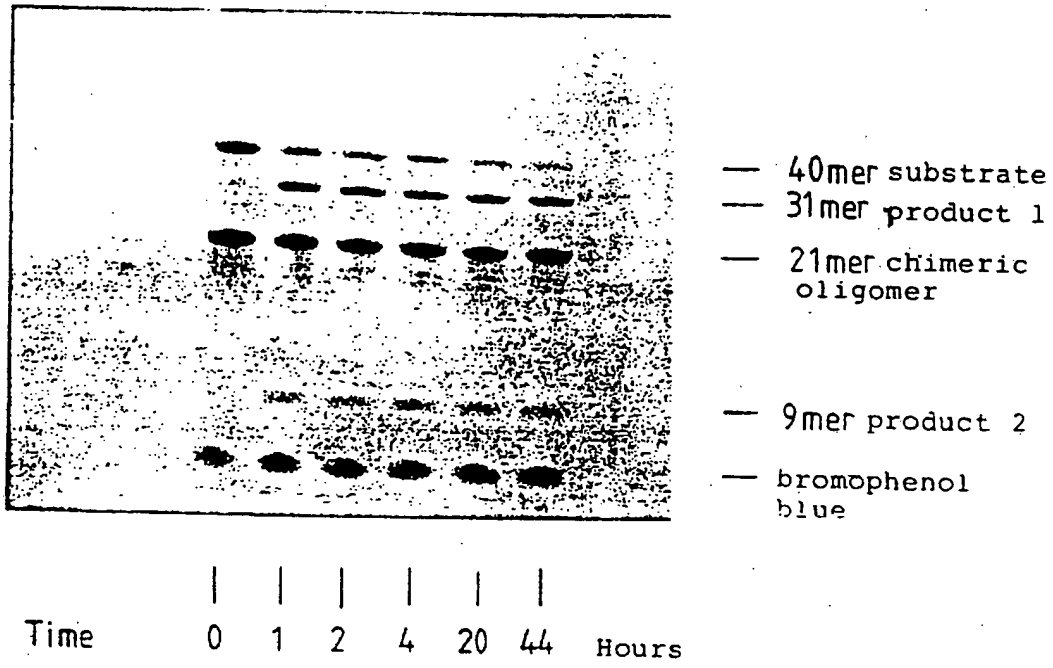

40mer substrate
31mer product 1
21mer chimeric oligomer
9mer product 2
bromophenol blue
Time
0 1 2 4 20 44
Hours

Fig. 4b

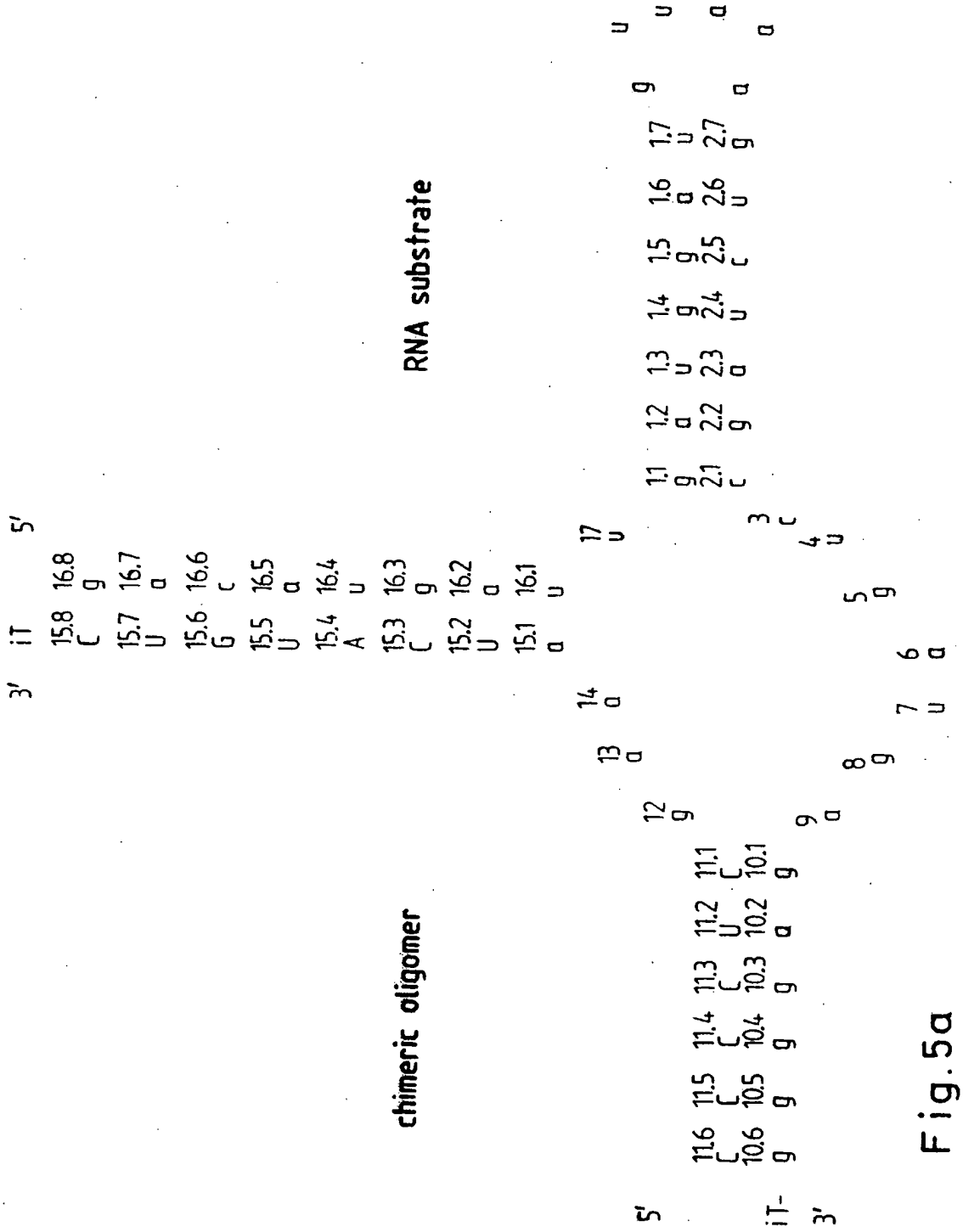

RNA substrate
chimeric oligomer
5'
3'
iT
15.8 16.8
15.7 16.7
15.6 16.6
15.5 16.5
15.4 16.4
15.3 16.3
15.2 16.2
15.1 16.1
17
1.1 1.2 1.3 1.4 1.5 1.6 1.7
2.1 2.2 2.3 2.4 2.5 2.6 2.7
3
4
5
14
13
12
9
8
7 6
11.6 11.5 11.4 11.3 11.2 11.1
10.6 10.5 10.4 10.3 10.2 10.1
iT-

Fig.5a

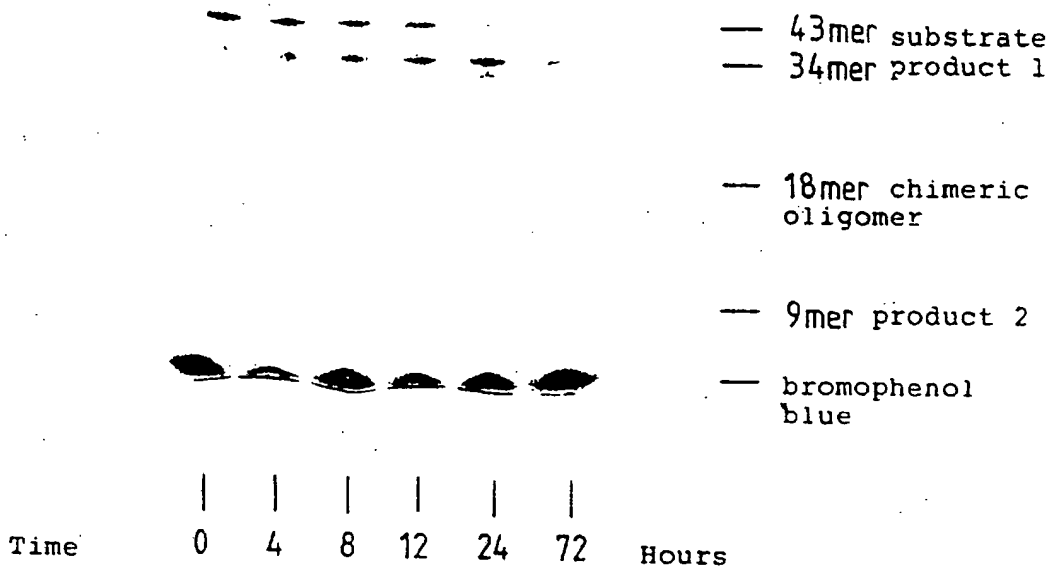

43mer substrate
34mer product 1
18mer chimeric oligomer
9mer product 2
bromophenol blue
Time
0
4
8
12
24
72
Hours

Fig. 5b

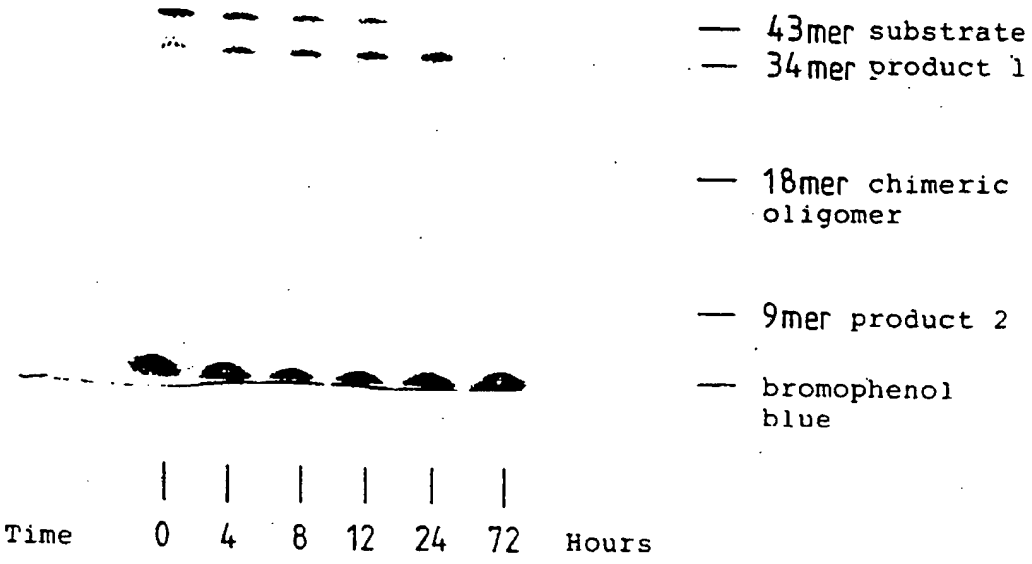

43mer substrate
34mer product 1
18mer chimeric oligomer
9mer product 2
bromophenol blue
Time
0
4
8
12
24
72
Hours

Fig. 5c

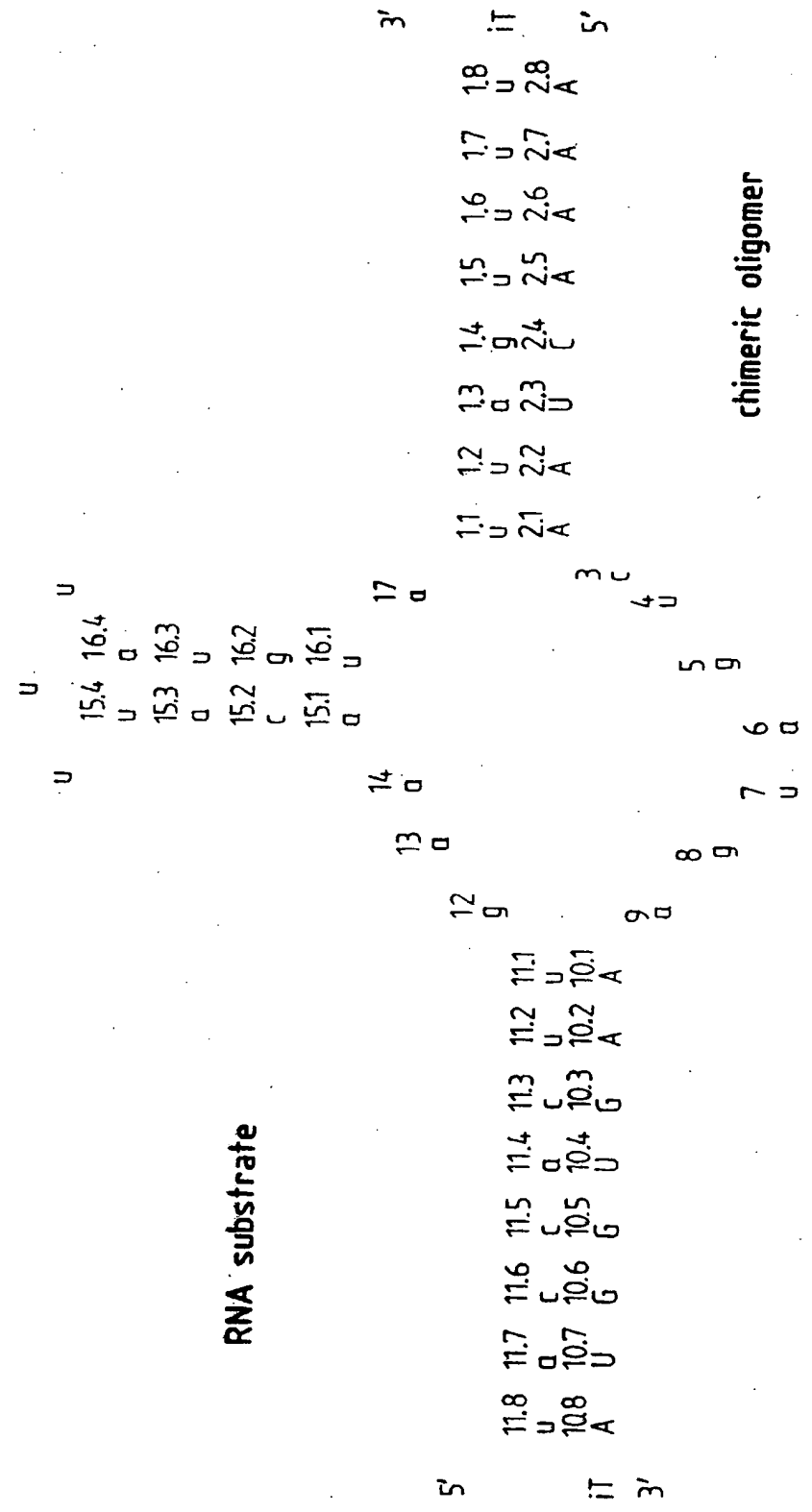


Fig. 6a

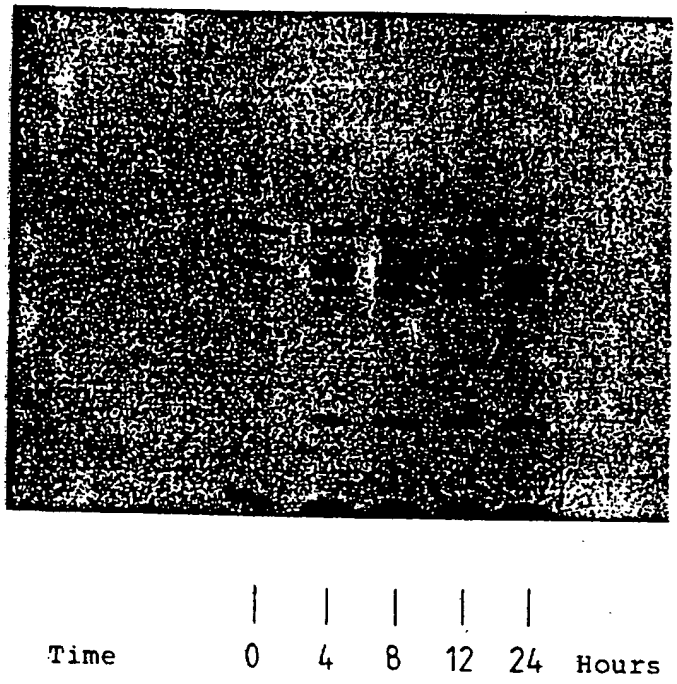

32mer substrate
24mer chimeric oligomer
23mer product 1
9mer product 2
bromophenol blue
Time
0
4
8
12
24
Hours

Fig. 6b

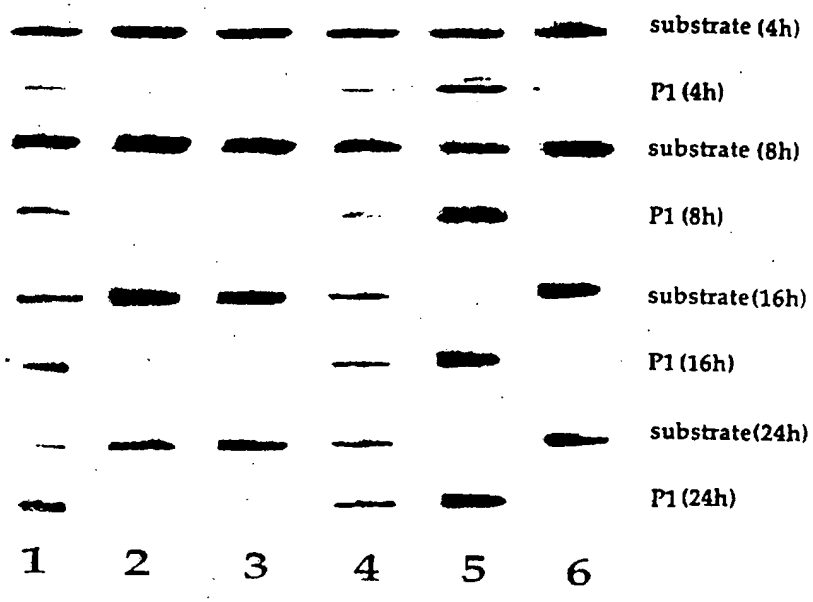

substrate (4h)
P1 (4h)
substrate (8h)
P1 (8h)
substrate(16h)
P1 (16h)
substrate(24h)
P1 (24h)
1
2
3
4
5
6

Fig. 6c

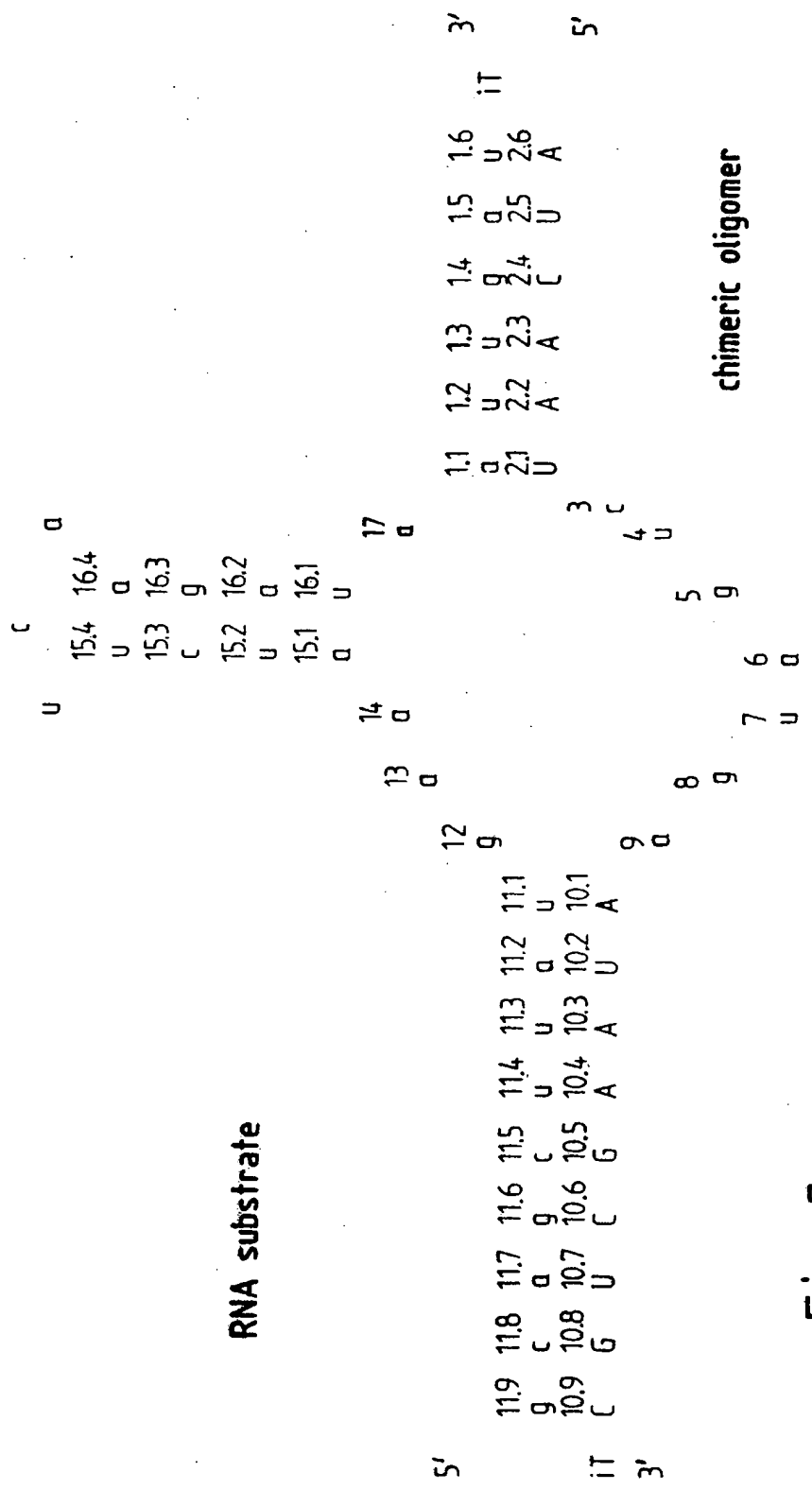


Fig. 7a

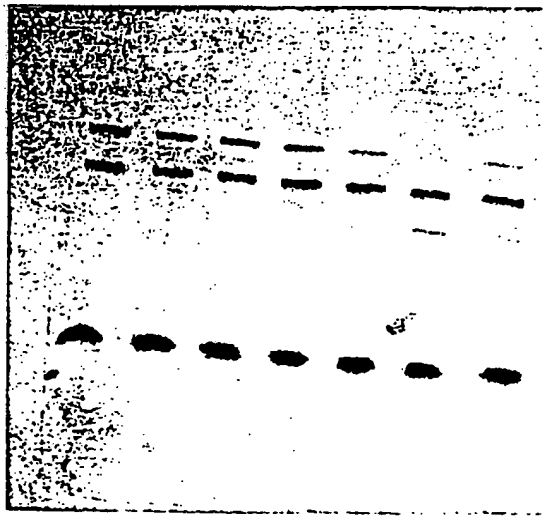

31mer substrate
23mer chimeric oligomer
24mer product 1
7mer product 2
bromophenol blue
Time 0 2 4 8 24 48 72 Hours

Fig. 7b

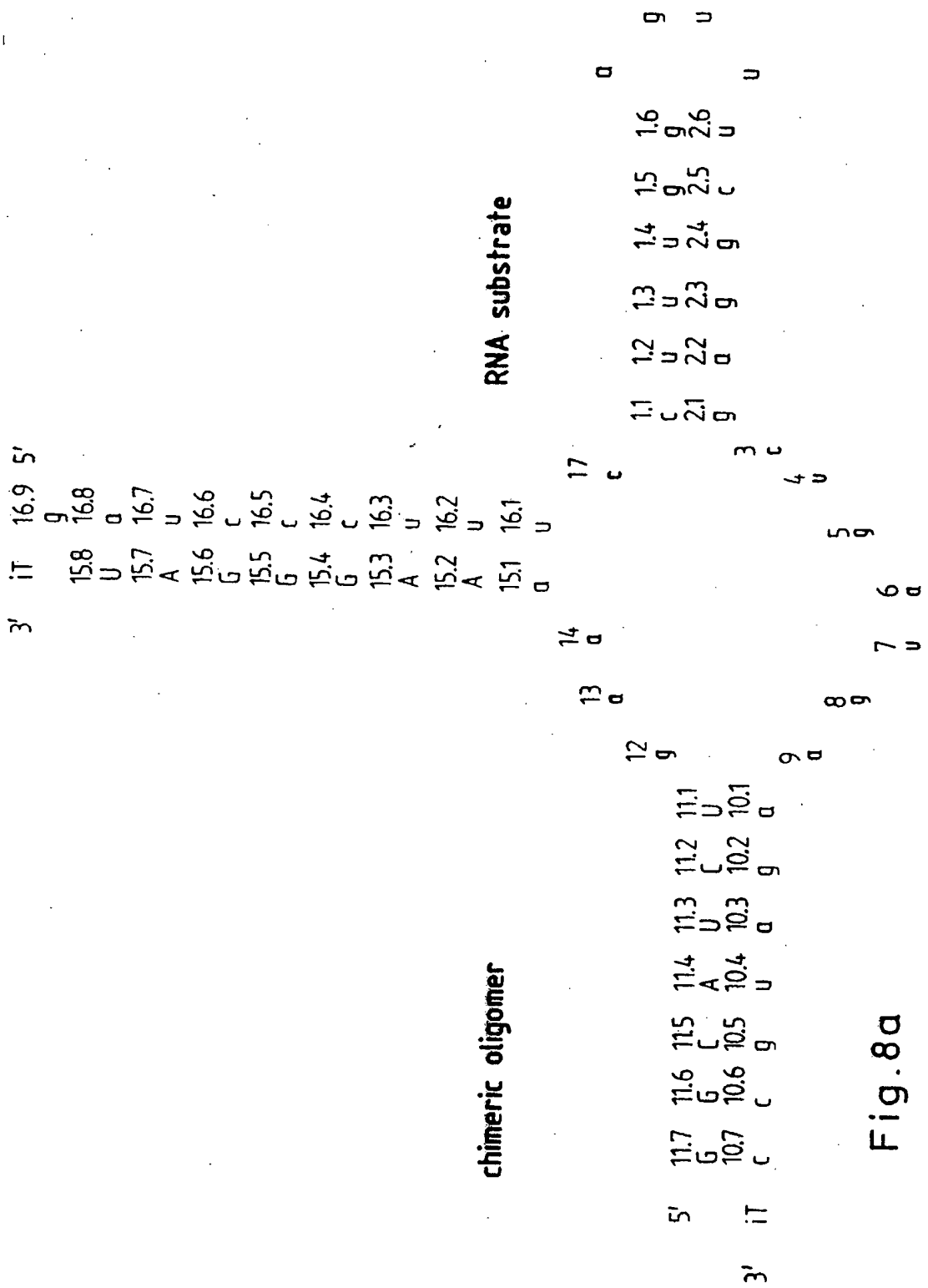

RNA substrate
chimeric oligomer
3' iT 16.9 5'
16.8
15.8
16.7
15.7
16.6
15.6
16.5
15.5
16.4
15.4
16.3
15.3
16.2
15.2
16.1
15.1
1.1
1.2
1.3
1.4
1.5
1.6
2.1
2.2
2.3
2.4
2.5
2.6
3
4
5
6
7
8
9
10.1
10.2
10.3
10.4
10.5
10.6
10.7
11.1
11.2
11.3
11.4
11.5
11.6
11.7
12
13
14
17
5' iT 3'

Fig.8a

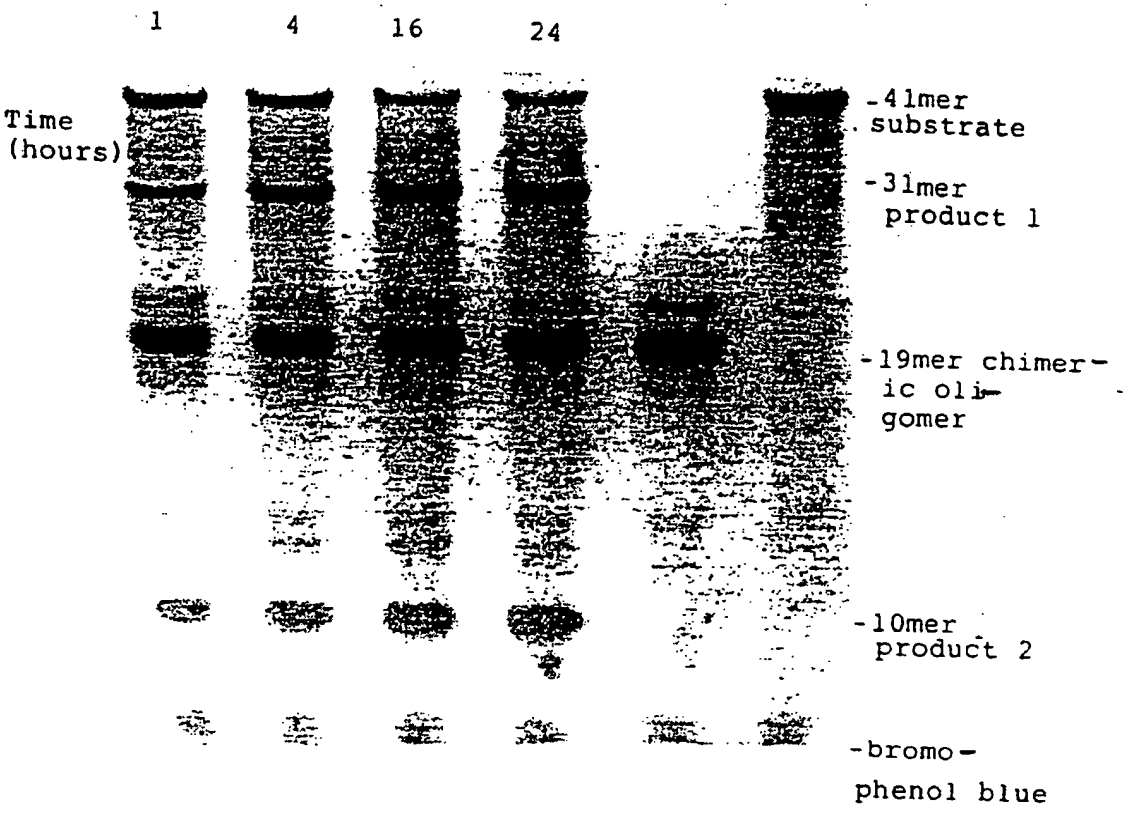
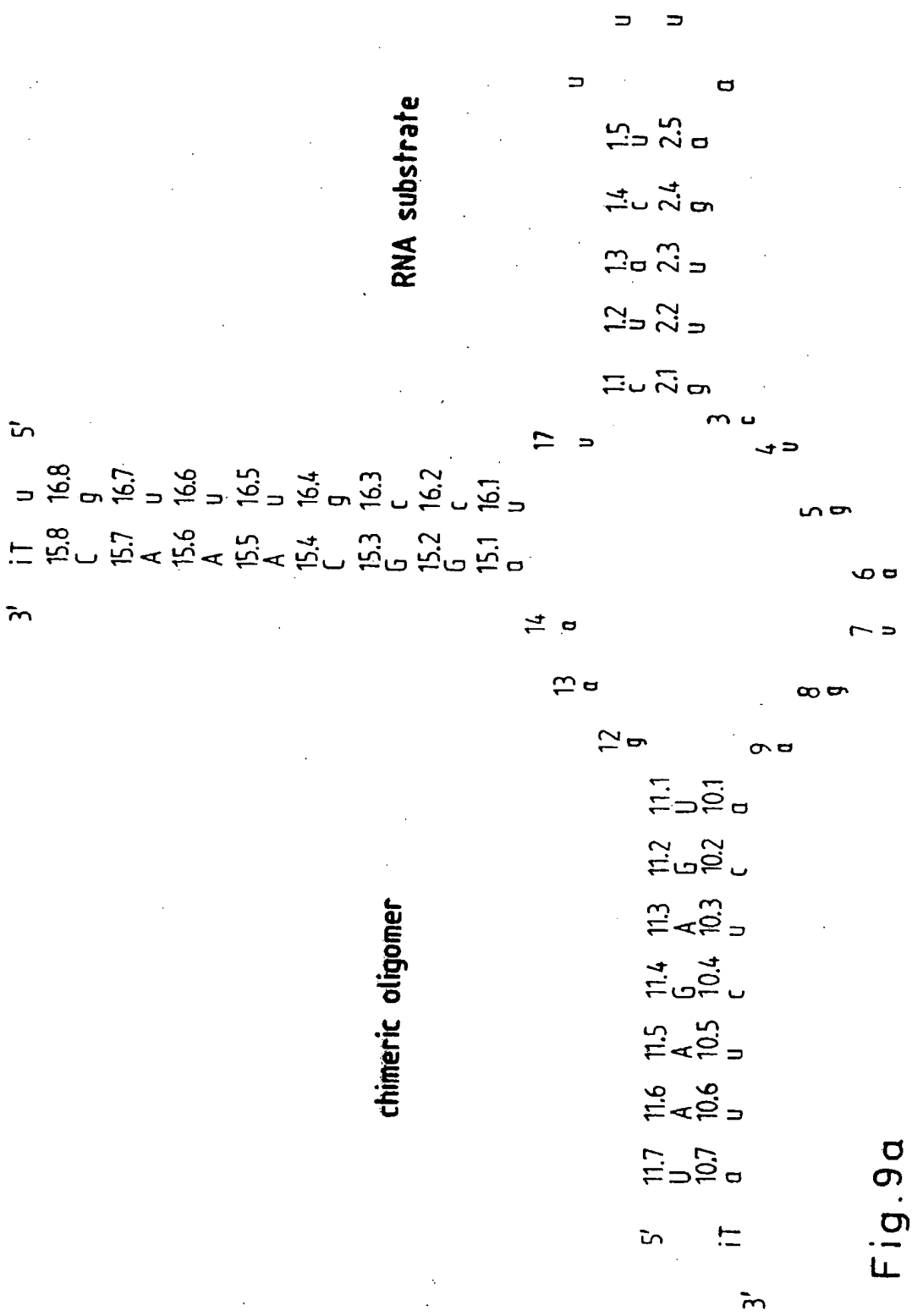

1
4
16
24
Time (hours)
-41mer substrate
-31mer product 1
-19mer chimeric oligomer
-10mer product 2
-bromophenol blue

Fig. 8b


RNA substrate
chimeric oligomer
5'
3'
iT
u
16.8
g
16.7
u
16.6
u
16.5
u
16.4
g
16.3
c
16.2
c
16.1
u
15.8
g
15.7
A
15.6
A
15.5
A
15.4
C
15.3
G
15.2
G
15.1
a
17
u
14
a
13
a
12
g
9
a
8
g
7
u
6
a
5
g
4
u
3
c
2.1
g
1.1
c
2.2
u
1.2
u
2.3
u
1.3
a
2.4
g
1.4
c
2.5
a
1.5
u
11.1
U
10.1
a
11.2
G
10.2
c
11.3
A
10.3
u
11.4
G
10.4
c
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A
10.5
u
11.6
A
10.6
u
11.7
U
10.7
a

Fig.9a

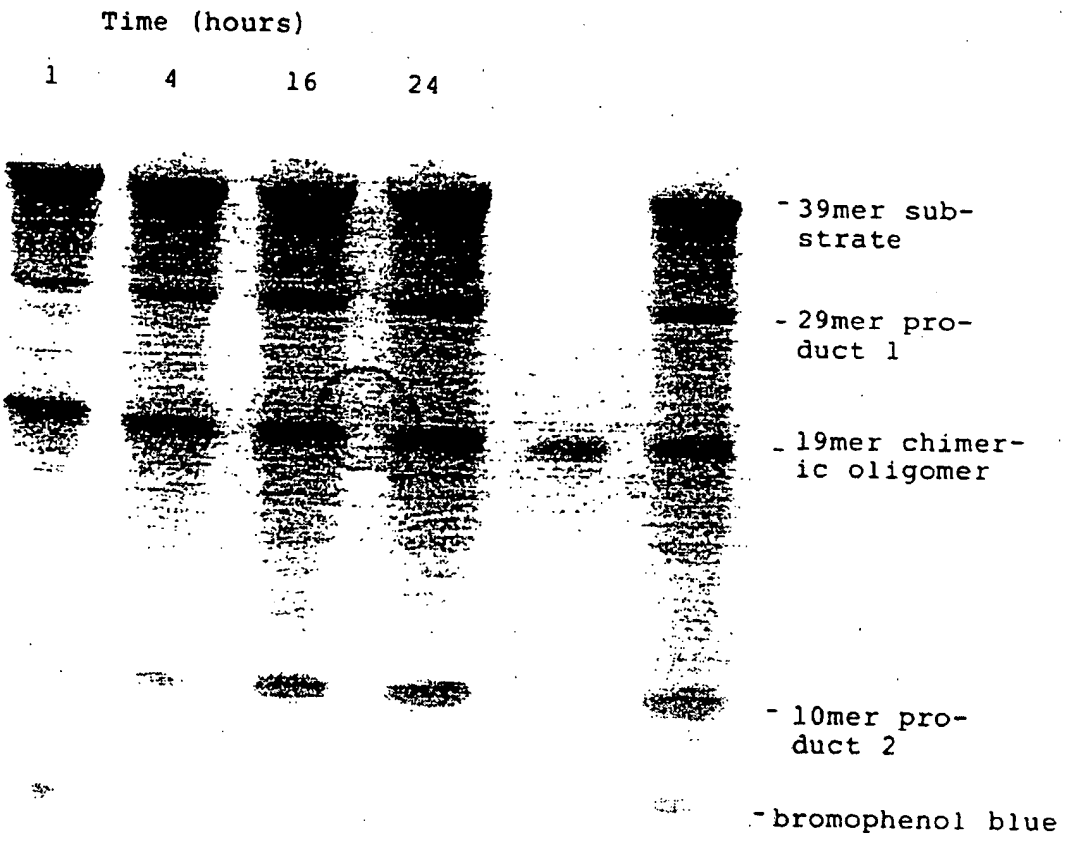
Time (hours)
1
4
16
24
- 39mer substrate
- 29mer product 1
- 19mer chimeric oligomer
- 10mer product 2
- bromophenol blue

Fig. 9b

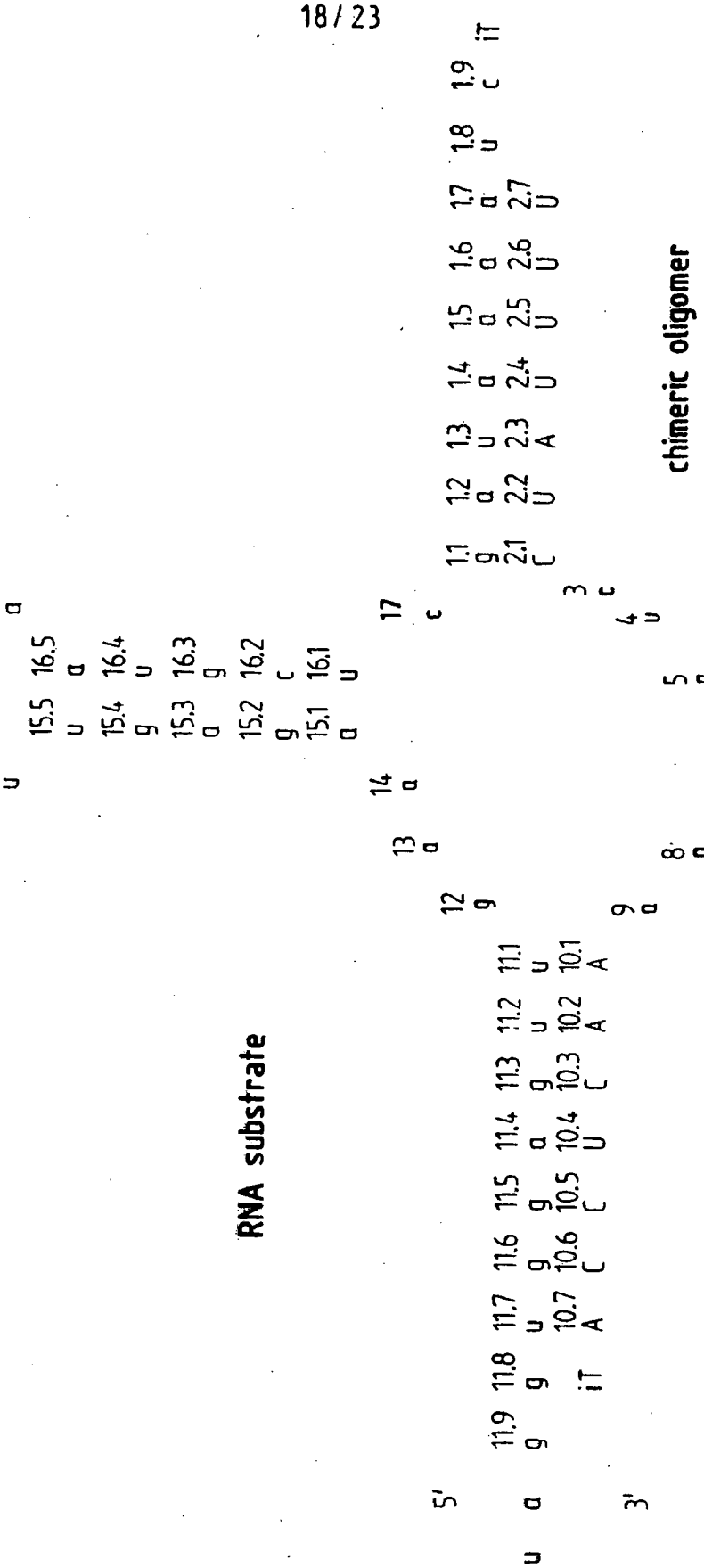

Fig. 10a

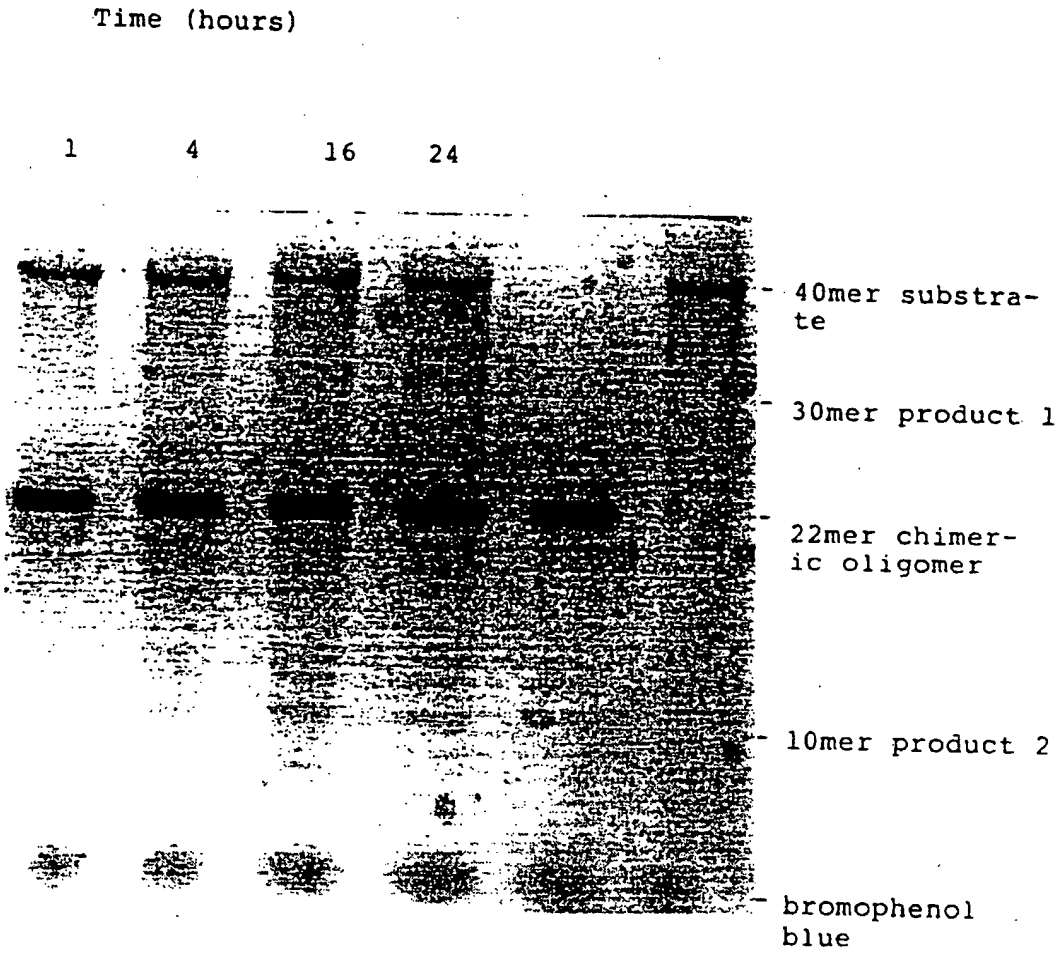
Time (hours)
1
4
16
24
40mer substrate
30mer product 1
22mer chimeric oligomer
10mer product 2
bromophenol blue

Fig. 10b

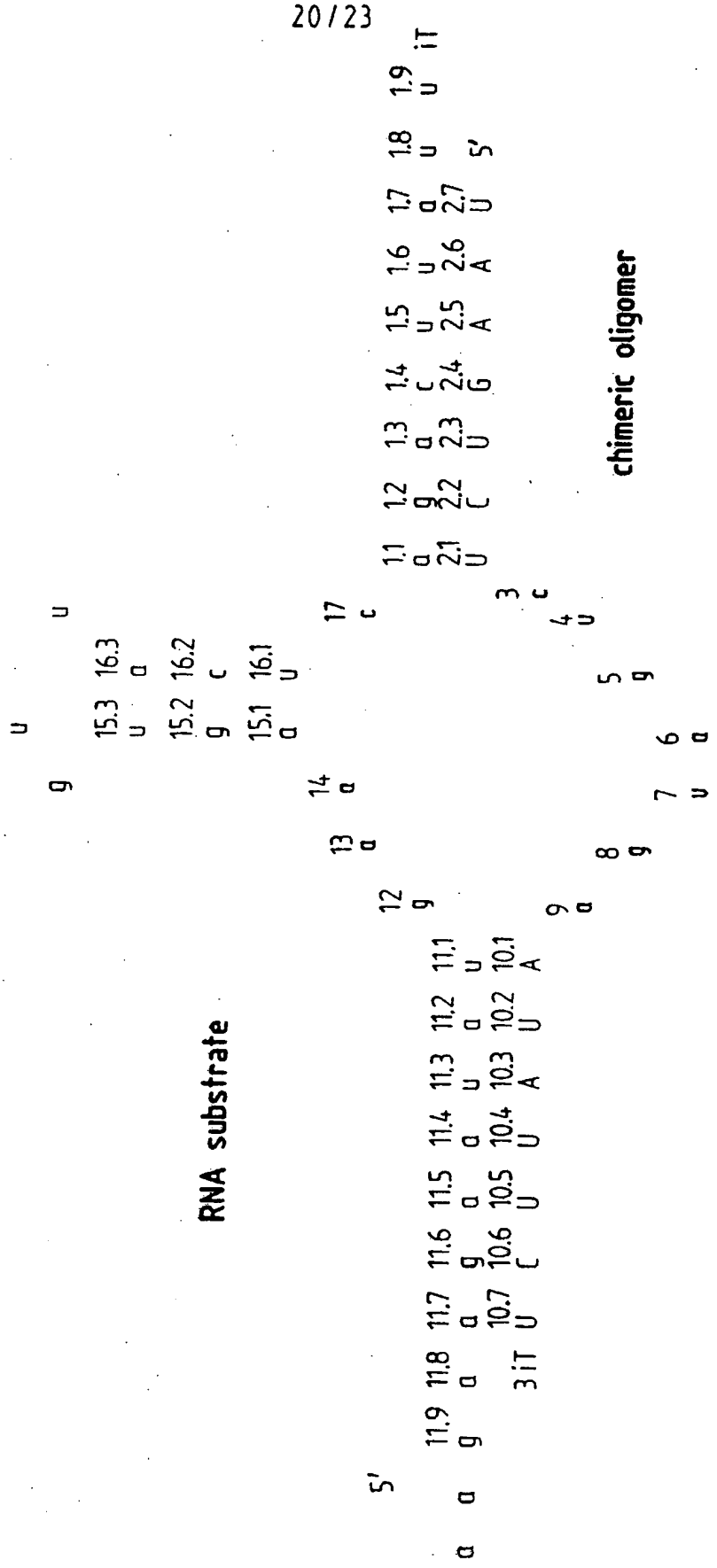
RNA substrate
chimeric oligomer
5'
3'T
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.1
2.2
2.3
2.4
2.5
2.6
2.7
3
4
5
6
7
8
9
10.1
10.2
10.3
10.4
10.5
10.6
10.7
11.1
11.2
11.3
11.4
11.5
11.6
11.7
11.8
11.9
12
13
14
15.1
15.2
15.3
16.1
16.2
16.3
17

Fig. 11a

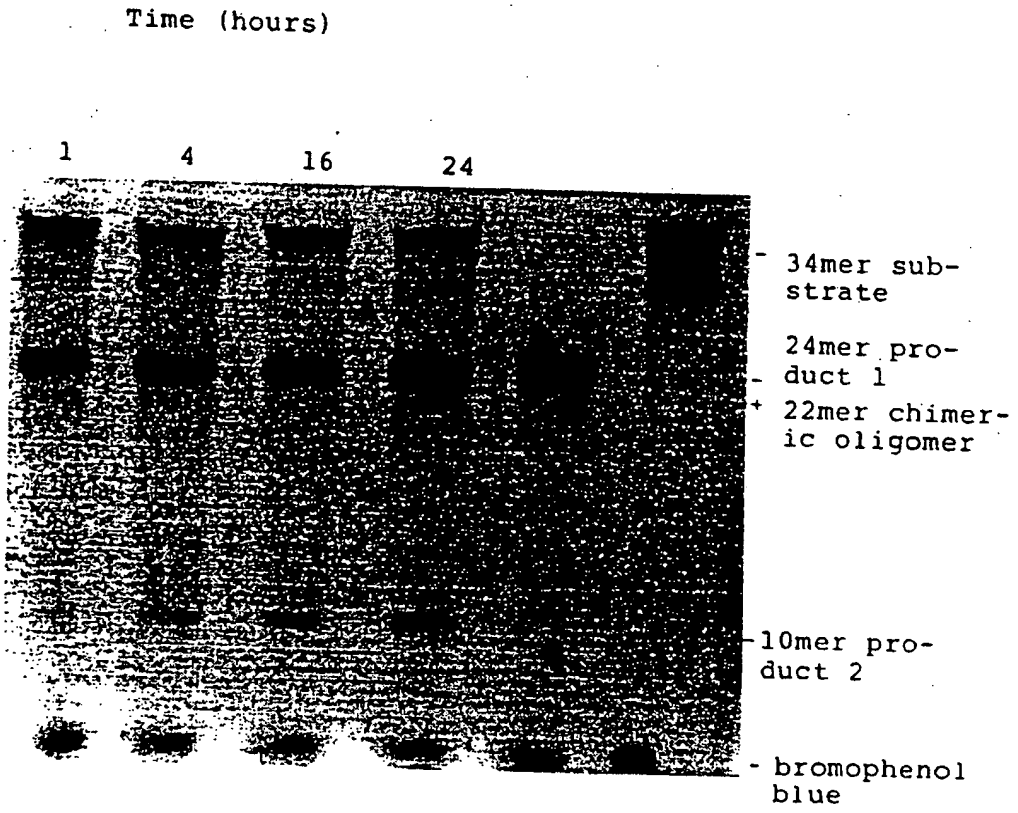
Time (hours)
1
4
16
24
34mer substrate
24mer product 1
22mer chimeric oligomer
10mer product 2
bromophenol blue

Fig. 11b

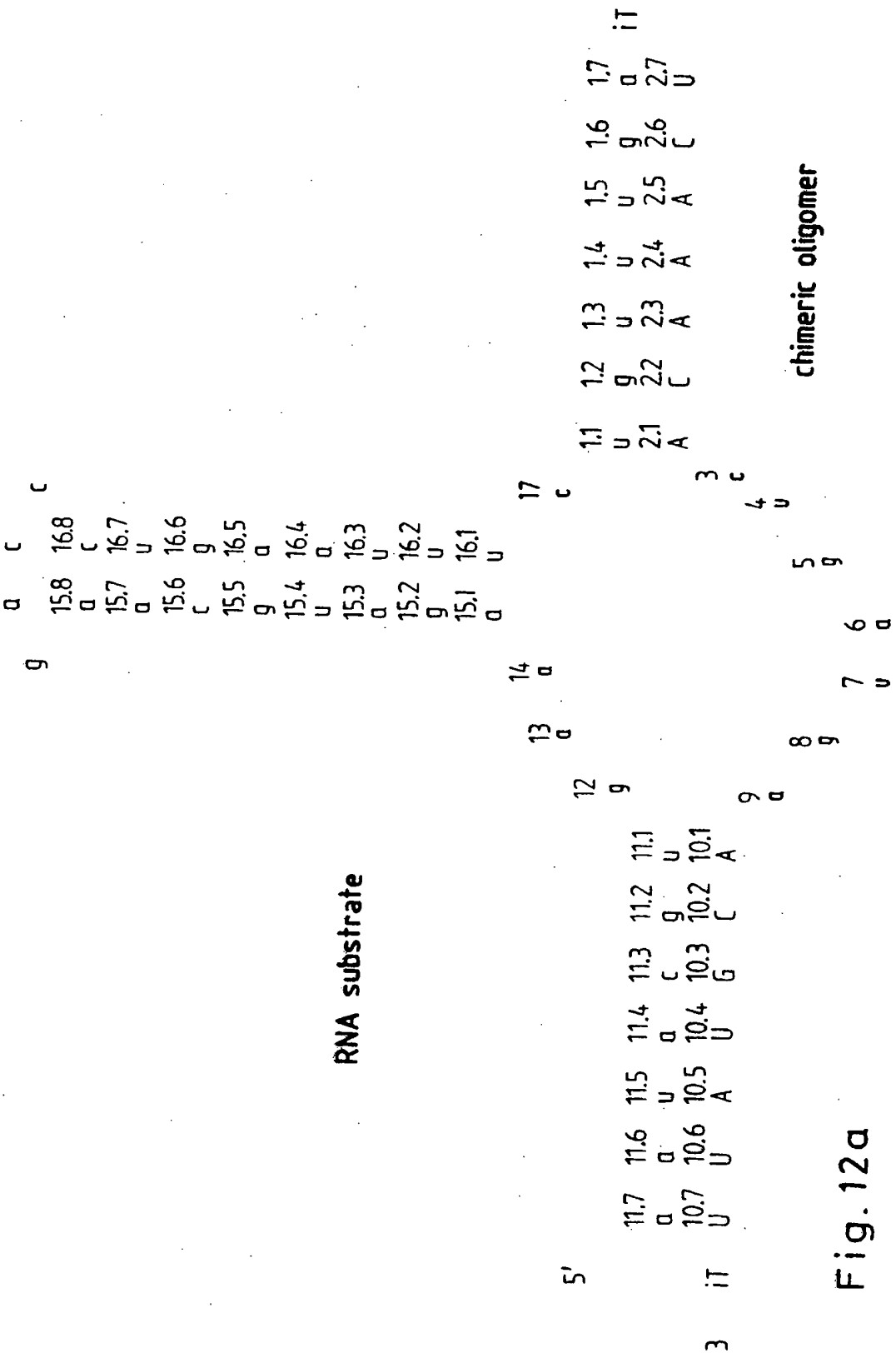
RNA substrate
chimeric oligomer
5'
3
iT
iT
a c c
g
15.8 16.8
a c
15.7 16.7
a u
15.6 16.6
c g
15.5 16.5
g a
15.4 16.4
u a
15.3 16.3
a u
15.2 16.2
g u
15.1 16.1
a u
14
a
13
a
12
g
9
a
17
c
3
c
4
u
5
g
8
g
7 6
u a
1.1 1.2 1.3 1.4 1.5 1.6 1.7 iT
u g u u u g a
2.1 2.2 2.3 2.4 2.5 2.6 2.7
A C A A A C U
11.7 11.6 11.5 11.4 11.3 11.2 11.1
a a u a c g u
10.7 10.6 10.5 10.4 10.3 10.2 10.1
U U A U G C A

Fig. 12a

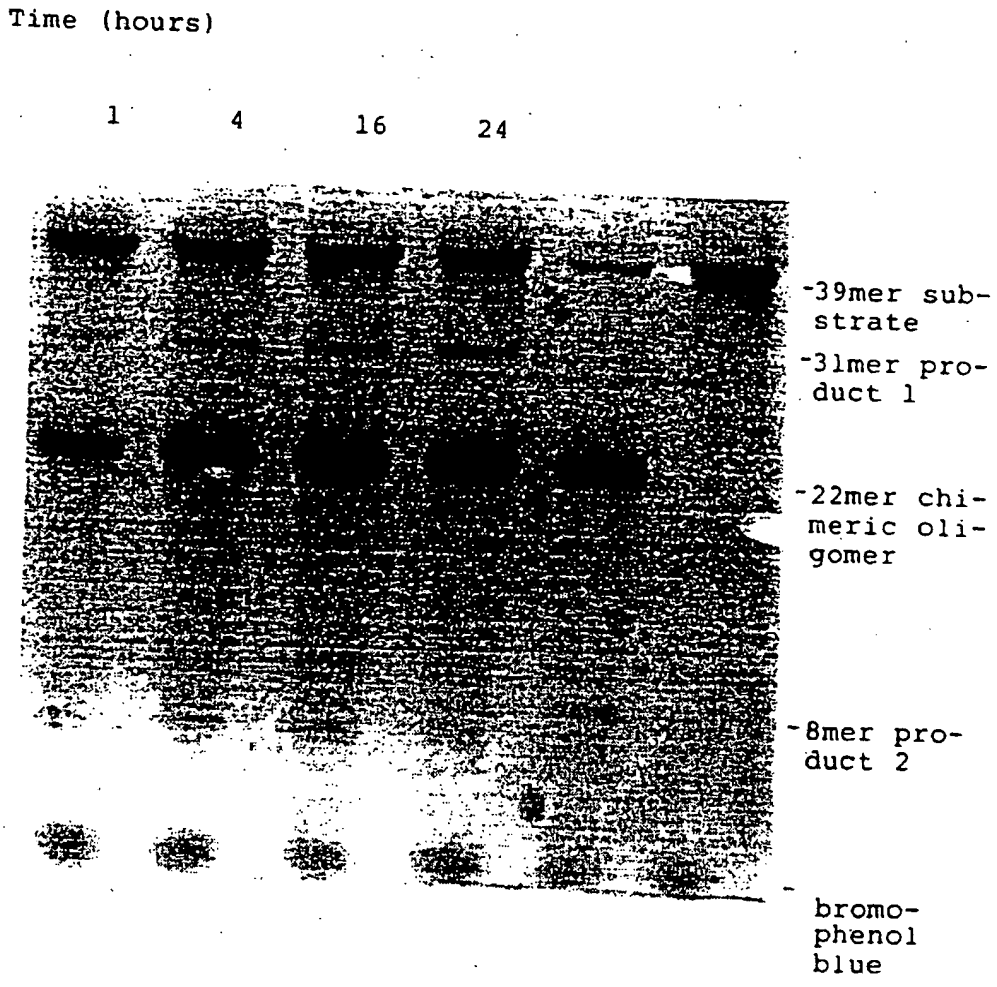
Time (hours)
1
4
16
24
-39mer substrate
-31mer product 1
-22mer chimeric oligomer
-8mer product 2
bromophenol blue

Fig. 12b

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/05014

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/52 C12N9/00 A61K31/70 C07H21/02 C12N15/11 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 95 11304 A (RIBOZYME PHARM INC) 27 April 1995 cited in the application see the whole document	1,9 2-8, 10-57

X Y	NUCLEIC ACIDS RESEARCH, vol. 17, no. 4, 25 February 1989, pages 1371-1377, XP002026788 A.C. JEFFRIES ET AL.: "A catalytic 13-mer ribozyme" cited in the application see the whole document	1,9 2-8, 10-57

Y	WO 94 10301 A (GENE SHEARS PTY LTD ;SIOUD MOULDY (NO)) 11 May 1994 see the whole document	1-57

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 1997	27.03.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	**Authorized officer** Hix, R

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 15187 A (MASSACHUSETTS INST TECHNOLOGY) 5 August 1993 see the whole document	1-57
Y	WO 91 19789 A (COMMW SCIENT IND RES ORG) 26 December 1991 see the whole document	1-57
Y	WO 92 07065 A (MAX PLANCK GESELLSCHAFT) 30 April 1992 see the whole document	1-57
Y	WO 94 13789 A (RIBONETICS GMBH ;LUDWIG JANOS (DE); BENSELER FRITZ (DE); KOTZOREK) 23 June 1994 see the whole document	1-57
A	THE EMBO JOURNAL , vol. 11, no. 4, April 1992, pages 1525-1530, XP000268587 P. STEINECKE ET AL.: "Expression of a chimeric ribozyme gene results in endonucleolytic cleavage of target mRNA and a concomitant reduction of gene expression in vivo" see the whole document	
A	NUCLEIC ACIDS RESEARCH, vol. 20, no. 17, 1992, pages 4559-4565, XP000644533 N.R. TAYLOR ET AL.: "Chimeric DNA-RNA hammerhead ribozymes have enhanced in vitro catalytic efficiency and increased stability in vivo" see the whole document	
A	THE EMBO JOURNAL, vol. 11, no. 5, May 1992, pages 1913-1919, XP000268586 G. PAOLELLA ET AL.: "Nuclease resistant ribozymes with high catalytic activity" cited in the application see the whole document	
A	J. AM. CHEM. SOC., vol. 115, 1993, pages 8483-8484, XP000644539 F. BENSELER ET AL.: "Hammerhead-like molecules containing non-nucleoside linkers are active RNA catalysts" cited in the application see the whole document	

-/--

INTERNATIONAL SEARCH REPORT

Inter tional Application No
PCT/EP 96/05014

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY 122 (4). 1996. 254-256. ISSN: 0171-5216, XP000618242 KOZU T ET AL: "Designing of chimeric DNA-RNA hammerhead ribozymes to be targeted against AML1-MTG8 mRNA." see the whole document ---	1-57

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 96/05014

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 52,53,58-63
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 52,53,58-63 as far as in vivo methods are concerned are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/ composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searches without effort jusufying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 96/ 05014

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1.) Chimeric oligomers with RNA-cleavage activity where the active centre has the formula 5'-GAAR-3' and the RNA-substrate contains a structure of the formula 5'-CUGANGAN-3' from **human interleukin-2 mRNA**, pharmaceutical compositions and uses thereof : Claims 1 to 17, 43 to 57 partially and Claims 18 and 19 completely.

2.) Chimeric oligomers with RNA-cleavage activity where the active centre has the formula 5'-GAAR-3' and the RNA-substrate contains a structure of the formula 5'-CUGANGAN-3' from **human ICAM-1 mRNA**, pharmaceutical compositions and uses thereof : Claims 1 to 17, 43 to 57 partially and Claims 20 and 21 completely.

3.) Chimeric oligomers with RNA-cleavage activity where the active centre has the formula 5'-GAAR-3' and the RNA-substrate contains a structure of the formula 5'-CUGANGAN-3' from **human PKC-α RNA**, pharmaceutical compositions and uses thereof : Claims 1 to 17, 43 to 57 partially and Claims 22 and 23 completely.

4.) Chimeric oligomers with RNA-cleavage activity where the active centre has the formula 5'-GAAR-3' and the RNA-substrate contains a structure of the formula 5'-CUGANGAN-3' from **human interleukin-1α mRNA**, pharmaceutical compositions and uses thereof : Claims 1 to 17, 43 to 57 partially and Claims 24 and 25 completely.

5.) Chimeric oligomers with RNA-cleavage activity where the active centre has the formula 5'-CUGANGAN-3' and the RNA-substrate contains a structure of the formula 5'-GAAR-3' from various sources, pharmaceutical compositions and uses thereof : Claims 1 to 8 and 43 to 57 partially and Claims 26 to 41 completely.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 96/05014

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9511304 A	27-04-95	AU 7978694 A	08-05-95
WO 9410301 A	11-05-94	AU 5412094 A	24-05-94
		EP 0672133 A	20-09-95
		JP 8505521 T	18-06-96
		ZA 9308208 A	03-01-95
WO 9315187 A	05-08-93	AU 3600193 A	01-09-93
		CA 2129119 A	05-08-93
		EP 0625192 A	23-11-94
		JP 7503613 T	20-04-95
WO 9119789 A	26-12-91	AU 650032 B	09-06-94
		AU 7997691 A	07-01-92
		CA 2084790 A	20-12-91
		EP 0535067 A	07-04-93
		JP 5509224 T	22-12-93
		US 5298612 A	29-03-94
WO 9207065 A	30-04-92	AT 147098 T	15-01-97
		AU 649074 B	12-05-94
		CA 2093664 A	13-04-92
		DE 69123979 D	13-02-97
		DE 552178 T	03-02-94
		EP 0552178 A	28-07-93
		ES 2061416 T	16-12-94
		JP 6501842 T	03-03-94
		PL 169576 B	30-08-96
WO 9413789 A	23-06-94	AU 5974194 A	04-07-94
		EP 0672122 A	20-09-95

QC - NATIONAL PHASE
(For APC and NP cases)

US PATENT
OUTGOING DOCKETING

CLIENT CODE: _____

AGENT: _____ **DATE:** _____

CHECKLIST INDICATORS: **Y = Yes** **N = No** **[#] = Number when Appropriate** **✓ = Not Applicable**

VERIFY ALL IS CORRECT

POSTCARD

_____ Today's Date.
_____ Express Mail Label number.
_____ Docket Number.
_____ Applicant information (et al.,).
_____ Title - ellipsis (. . .) used when title is incomplete.
_____ Initialed by the Assistant.
_____ Attorney initials indicated.
_____ Number of pages and/or sheets appear where required.
_____ # of claims paid for entered in claim space.
_____ Filed Signed _____ ; or Unsigned _____.
_____ Fee amount matches check and transmittal.
_____ All enclosures are marked.
_____ Initial postcard at **QC:** once verified.

CHECK (if applicable)

_____ Fee entered as calculated on transmittal letter.
_____ Docket Number and 1st named inventor are listed in memo section.
_____ Numerical and written amount are the same.
_____ Check is dated.
_____ Sign check.

EXPRESS MAIL CERTIFICATE

_____ Addressed to:
Mail Stop PCT, Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450.
_____ Docket Number.
_____ Inventor information (et al., or listed).
_____ Title is complete and correct.
_____ Attorney or Agent Name.
_____ Express Mail Label number.
_____ Date of Deposit is correct.
_____ All enclosures are listed.
_____ Signed.

TRANSMITTAL

_____ Heading is complete and includes:
____ Docket number.
____ First named Inventor is provided and correct.
____ Full title.
____ International Application Number.
____ International Filing Date.
____ Claimed Priority Date.
____ Express Mail Label number.
_____ Date appearing in header reflects filing date.
_____ Full name of each Inventor is provided and correct.

TRANSMITTAL (continued)

_____ **Application** - choose appropriate and complete:
____ Not required - Filed in US Receiving Office.
____ Transmitted by International Bureau (IB).
____ Form PCT/IB/308 enclosed (not required).
____ Attached - Copy as **Originally** Filed. DOES NOT APPLY TO Published PCT Application **or** English Translation.
____ Title is complete and correct.
[___] Total Claims
[___] Total Independent Claims
[___] Consecutively numbered pages.
[___] Sheets of Drawings.
____ Drawing sheets are in numerical order.
____ Application information on drawings.
____ All figures are mentioned in the specification.
Number of figures [_____].
____ Sequence Listing in [_____] pages.
____ Appendices in [_____] pages.
____ Application Size in [_____] pages. **Add all above sheet and page totals.**

_____ **Article 19 Amendments** - choose appropriate and complete:
____ Have not been made - no enclosure.
____ Communicated by International Bureau (IB).
____ Form PCT/IB/337 enclosed (not required).
____ Attached.
[___] Consecutively numbered pages.

_____ **English** translation of specification (may exclude drawings).
[___] Consecutively numbered pages.
_____ All enclosures are listed.
_____ Number of pages and/or sheets appear where required.
_____ Filing fee is correctly calculated based on Claims, Search type, and Examination type indicated in Fee Table.

NOTE: Search and Examination fees have been removed from table below because we must take the attorney's word for which fee amount is required.

_____ Multiple Dependent claims?

____ **Small** Entity Fee - Transmittal must include statement: "The present application qualifies for Small Entity ..."

____ **LARGE** Entity Fee:

Fee Type	Small	Large
Filing	150	300
Total Claims in excess of 20	25	50
IND Claims in excess of 3	100	200
Multiple Dependent Claims	180	360
Application Size *(when enclosed)* Spec, Claims, Abstract & Drawings (Each group of 50 pgs over 100)	125	250

_____ Signed by Attorney or Agent.

PRELIMINARY AMENDMENT (if applicable)

_____ Docket number.
_____ Inventor information (et al, or listed)
_____ Full title.
_____ Priority claim appears as first paragraph under the heading, "Related Applications."
[_____] Consecutively numbered pages.
_____ Signed and dated.

SUBSTITUTE SPECIFICATION (if applicable)

_____ Docket number.
_____ Full title.
_____ Priority claim appears as first paragraph under the heading, "Related Applications."
_____ Title on Abstract page is correct (if given).
[_____] Consecutively numbered pages.

DECLARATION (if applicable)

_____ Docket number.
_____ Full title.
_____ Herewith is entered as the filing date.
_____ Unknown is entered as the serial number.
_____ All fields for priority information are complete for each item.
_____ PCT application listed in US section.
_____ Foreign priorities listed in foreign section.
____ Priority claim indicated as "Yes" or "No" for each application.
_____ Each inventor has signed where indicated.

ASSIGNMENT (if applicable)

_____ Assignment document signature(s) dated **same day or after** Declaration.
____ Order of inventors matches Declaration.
_____ Recordation Form Cover Sheet is complete:
____ Order of inventors matches Declaration.
____ Assignee name matches Assignment.
____ Each signature date is listed when multiple dates.
____ Docket number.
____ Total number of applications = 1.
____ Signed & Dated by Attorney/Agent.
____ Attorney/Agent's registration number is listed.
____ Total number of pages **includes cover sheet**.
_____ **$40** Recordation Fee.
____ Included in filing fee; or
____ Separate check.

INFORMATION DISCLOSURE STATEMENT (if applicable)

_____ Docket number.
_____ Inventor information (et al., or listed).
_____ Title is correct and complete.
_____ Search Report listed on PTO/SB/80 equivalent.
_____ Search Report enclosed.
_____ Foreign References and Non-Patent Literature enclosed.
Total listed references [_____].
Enclosed references [_____].
_____ Signed and dated.

POWER OF ATTORNEY (if applicable)

_____ KMOB identified by Customer Number or **Irvine Address.**
_____ Attachments stamped **COPY DO NOT RECORD.**

OTHER ENCLOSURES

_____ Docket number **on each enclosure.**
_____ Inventor information (et al., or listed) **on each enclosure.**
_____ Title is correct and complete **on each enclosure.**
_____ All other enclosures are listed on the **transmittal.**
_____ All other enclosures are listed on the **postcard.**
_____ Sequence Listing enclosed?
____ Signed Sequence Submission Statement.
____ Disk with computer readable format (CRF) copy of Sequence Listing.

EXPRESS MAIL ENVELOPE & EXPRESS MAIL LABEL

_____ Proper sized Express Mail envelope is being used.
_____ Addressed to:
Mail Stop PCT, Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450.
_____ Docket Number entered correctly on label.
_____ Attorney initials appear on the label.
_____ Place PTO documents in Express Mail envelope.

FILE COPY

_____ File copy is complete & secured in center of file.
- Includes copy of check, postcard, drawings, transmittal, application, all other enclosures.

_____ Initial copy of postcard.
_____ Sign copy of check.

LETTER TO CLIENT

_____ Docket number is correct.
_____ Title is correct and complete.
_____ Addressee on letter is the same as the envelope.
_____ Proper sized envelope is being used.
____ Docket Number entered correctly.
____ Attorney initials appear.
_____ Place Client Copy in envelope.

[Continued on Next Page]

PATENT MANAGEMENT SYSTEM
Verify and correct information

COUNTRY APPLICATION DATA

Launch **Patent Docketing**; Click the **Country Applications Data** button; Enter the entire file name in the **Case Number** field; Click the **Start Search** button.

_____ Country = **US**.

_____ Type = **APC**.
Please note that **NP cannot be used** as it stands for a filing in Nepal.

_____ Filing Date = Date application mailed to PTO.

_____ Small Entity? (***See*** Transmittal) Change to **SE.**

_____ Click **SAVE** button

_____ Click on **Remarks** and add each of the following:

____ "[Mailing date] is date of Entry into National Phase; Filing date to be confirmed on Notice of Acceptance."

____ **Enter Priority Information.** ***See*** Continuity and Foreign Continuity sections on Transmittal letter. Enter priority information as listed on transmittal by typing in remarks:

- Priority from [priority application number]; Filed [date]
- **Ex:** Priority from 60/123,456 Filed 1/1/02.

____ Pull up the Country Application Data for each application number listed and add the present application to remarks by typing:

- Related to [this docket number]

_____ Click **SAVE** button

_____ Click **Invention** button

INVENTION SCREEN

_____ Disclosure Status = **Filed**.

_____ At **Client:** verify alpha code (e.g., **CLIENT**.001A).

_____ Change listed title if different than filed documents.

____ Pouch extra copy of face sheet to **Accounting-Title Change.**

_____ Click **SAVE** button

_____ Under **Inventors:** verify or enter each inventor in order using:

____ Declaration (1st choice)

____ Application Data Sheet (2nd choice)

____ Transmittal letter (3rd choice)

_____ Incomplete inventor names in list:

- Click **Info:**
- Enter FULL inventor name in the **Greeting** field.

_____ Too many inventors in system.

- e-mail Tina Han to delete inventor(s).

_____ Click **SAVE** **PRINT** **EXIT** buttons

_____ Click **List of Actions** tab

DOCKET INSTRUCTION SHEET

_____ Signed by Working Attorney/Scientist? **Must be signed.**

I. REMOVE DOCKET DATES

_____ Signed by partner? **Cannot remove dates without partner approval.**

_____ No dates selected for closure.

_____ Close date and annotate remarks by typing: *Per [attorney's 3 initials] close priority claim date [date on checklist].*

II. ADD DRAWING DATES

Box a - Calculate the later of 14 months from priority or 1 month from filing and add appropriate action.

_____ US-PAT DRAWING REMIND 14 MON

_____ US-PAT DRAWING REMIND 1 MON

Box b

_____ US-PAT DRAWING REMIND 6 MON

Box c

_____ US-PAT DRAWING REMIND 2 MON

III. FOREIGN FILING DOCKET DATES

NOTE: *Section III does not apply for National Phase applications.*

IV. RELATED APPLICATIONS

_____ **For each matter,** add each related docket number in general remarks, may be in addition to added priority info. Example:

- Related to [docket number(s)].

_____ Attach Instruction Sheet to checklist.

LIST OF ACTIONS

_____ **CLOSE:** NATL U.S. APPLN DUE or similar action.

____ Response sent date = mailing date.

____ Click **SAVE & EXIT** buttons

ASSIGNMENT (if applicable)

_____ **ADD:** US-PAT MD ASSIGNMENT

____ Action Base Date = mailing date.

____ Add to Remarks:

- Via Express Mail.
- For EACH Assignment enter: [Assignee] to [Assignor].

_____ Click **SAVE** **PRINT** **EXIT** buttons

Complete section below appropriate for **Application Filed With:**

1) **Declaration & ALL Fees:** Application filed with a fully signed declaration and <u>all</u> filing fees (Basic Filing Fee, Search Fee, and Examination Fee).

2) **No Declaration and/or Missing Fees:** Application filed without a signed declaration and/or <u>without all</u> filing fees.

1) DECLARATION & ALL FEES

_____ **ADD:** US-PAT MD NPHASE

____ Action Base Date = mailing date.

____ List all enclosures in remarks (exclude postcard, check and express mail certificate):

- Spec in # pgs; Decl in # pgs; [Item] in # pgs; etc.

_____ IDS being filed?

____ **CLOSE:** IDS w/Search Report Refs and Reminder

____ Response sent date = mailing date.

____ Remarks: Indicate if Search Report was submitted and/or if listed references came from a Search Report.

- IDS [including Search Report] w/# Refs [cited on Search Report], # Refs listed.

_____ Click **SAVE** **PRINT** **EXIT** buttons

2) NO DECLARATION and/or MISSING FEES

_____ **ADD:** US-PAT MD NPHASE MPART

____ Action Base Date = mailing date.

____ Remarks = "[Mailing date] is date of Entry into National Phase; Filing date to be confirmed on Notice of Acceptance."

____ List all enclosures in remarks (exclude postcard, check and express mail certificate):

- Spec in # pgs; Decl in # pgs; [Item] in # pgs; etc.

_____ IDS being filed?

____ **CLOSE:** IDS w/Search Report Refs and Reminder

____ Response sent date = mailing date.

____ Remarks: Indicate if Search Report was submitted and/or if listed references came from a Search Report.

- IDS [including Search Report] w/# Refs [cited on Search Report], # Refs listed.

_____ Click **SAVE** **PRINT** **EXIT** buttons

FACE SHEET

_____ PRINT face sheet.

_____ Double check your work product.

_____ Place in plastic sleeve on the front of the file.

_____ Fasten the Checklist to the top of the file.

LOG FILE OUT TO **DOCK** AND SHELF FOR AUDIT TRAIL

Audit Agent: _____

Date: _____

INDICATORS: **Y = Yes** **N = No** **✓ = Not Applicable**

NOTE: Do not use an indicator if there are errors, rather use the indicator once the errors have been corrected.

COUNTRY APPLICATION DATA

_____ Country = **US**.

_____ Type = **APC**.

_____ Filing Date = Date application mailed to PTO.

_____ Entity Status complete.

_____ Priority information appears in remarks:

- **Ex:** JONES.001PR, 60/123,456 Filed 1/1/02.

INVENTION SCREEN

_____ Disclosure Status = **Filed**.

_____ Check **Client:** (e.g., **CLIENT**.001A).

_____ Title in docketing matches filed documents.

_____ Under **Inventors:** verify full names of inventors appear:

____ Full name in Inventor Field; or

____ Full name in Inventor Table.

LIST OF ACTIONS

Verify section below appropriate for **Application Filed With:**

1) **Declaration & ALL Fees:** Application filed with a fully signed declaration and all filing fees (Basic Filing Fee, Search Fee, and Examination Fee).

2) **No Declaration and/or Missing Fees:** Application filed without a signed declaration and/or without all filing fees.

1) Declaration & Fees

_____ Action Base Date = mailing date.

____ US-PAT MD NPHASE

2) **NO** Declaration and/or Fees

_____ Action Base Date = mailing date.

____ US-PAT MD NPHASE MPART

____ Remarks = "[Mailing date] is date of Entry into National Phase; Filing date to be confirmed on Notice of Acceptance."

OTHER ACTIONS under List of Actions

_____ Action Base Date = **Mailing Date** for CLOSED actions.

_____ NATL U.S. APPLN DUE or similar action is CLOSED.

_____ IDS filed?

____ IDS dates are CLOSED

____ Remarks indicate if Search Report or references from the Search Report were listed.

_____ **Verify additional CLOSED dates are authorized by partner on Docket Instruction Sheet.**

- **Re-Open dates when NO Partner Instructions.**

_____ Assignment Filed?

____ Notice of Recorded Doc.? is OPEN.

_____ All enclosures are listed in remarks:

- Spec in # pgs; Decl in # pgs; [Item] in # pgs; etc.

_____ Other OPEN dates per Docket Instruction Sheet.

_____ ALL requests in Docket Instruction Sheet have been entered.

_____ **Fasten checklist to center of file.**

_____ **Log out to appropriate extension.**